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Proceedings of the 23 rd General Meeting of Japanese Biochemical Society

(*at Kobe May 3~5, 1952*)

1. A new type of glass electrode and its application to biological meterial. By TERUO ASADA (*From the Department of Physiology, Kyoto Prefectural Medical Collegge, Kyoto*). The author has devised a new glass-electrode for the titration of biological fluid. By use of this apparatus, the titration can be performed with a few ml. of sample solution in the presence of volatile acids, such as carbonic or bicarbonic acids. The differential titration after Yoshimura is carried out with this electrode as follows: (a) Original urine is titrated; (b) the urine is mixed with $Ba(OH)_2$, the formed precipitates is centrifuged off, and the supernatant solution is titrated, successively. From the difference of these two titration curves, the amounts of acid or base radicals can be calculated.

2. Thermo analysis of biochemical reactions. I. By HIROSHI FUNAKI (*From the Institute of Biological Physiochemistry, Kyoto Prefectural Medical College, Kyoto*).

3. A micromethod for the determination of the bound water of blood. By KAICHIRO KURODA and TETSUICHI NAKANISHI (*From the Department of Biochemistry, Tokushima University Medical School, Tokushima*). A micromethod has been divised for the determination of the bound water, based on the same principle of the authors' capilary method. The method consists in filling up a small capillary glass tube with a small amount of blood from an ear lobe, putting it in a little larger glass tube, one end of which has been closed beforehand, and then closing up the open end of the sheath by melting it over a small flame of an alcohol lamp. The difference in the weight of the tare and these tubes with the content gives the fresh weight. In a nickel box (92 mm. × 8 mm. × 10 mm.), the glass tube is broken to pieces by presing the box with another smaller box. The nickel boxes are placed in a saturated NaCl-desiccator, and the water is evaporated at 30°C *in vacuo.* for 24 hours. The evaporated water gives the amount of free water. Then the sample is placed in an electric oven kept at 95—100° and dried over 24 hours. The total loss of weight in 2 stages of evaporations represents the total water. The bound water can be now calculated: Bound water = total water-free water. Using this micromethod, the amounts of bound water of human blood, are found as follows: whole blood, $4.33 \pm 0.118\%$; serum, $2.56 \pm 0.114\%$; blood cells, $4.61 \pm 0.209\%$.

4. Studies on the foaming phenomena of human saliva, urine and blood. By ATSUSHI OSHIKANE (*From the Department of Biochemistry, School of Dentistry, Nippon University, Tokyo*). The foaminess and its stability of human salivary secrets, (normal, dietary, and pilocarpin stimulated), urine and blood were studied under various conditions of the subjects.

5. On the formation of urinary stones. III. By ZIRO NAGASAWA (*From the Department of Medical Chemistry, Kurume Medical College, Kurume*).

6. Pleolysis of red cells of various animal species. By MAKOTO MIYAZAKI and MINORU MASUDA (*From the Department of Biochemistry, Yokohama University Medical School, Yokohama*). The red cell suspension in aqueous solutions of neutral salts of consecutive concentrations shows the maximum hemolysis at a certain definite concentration; this is the so-called "pleolysis." Pleolysis can be revealed by the following facts: (a) the maximum lucidity of the suspension; (b) the maximum hemolysis; (c) the minimum tint, and (d) the minimum size of the centrifuged sediment of the suspension. By examining these 4 items, the experiments were carried out to evaluate the characteristic concentration upon the red cells of 33 different animals. With these materials, hemolysis was operated under the definite conditions (erythrocyte concentration, time, temperature and mechanical active mass fixed). By comparing the physical properties from (a) to (d), it has been found that the ploelysis is a universal phenomenon. It was concluded that (1) pleolysis can be most clearly observed in the property of the centrifuged sediments, especially in tint; (2) the pleolytic concentration is found to be in the vicinity of 0.05 M with the neutral salts of the type 1–1 (*e.g.*, NaCl); and (3) the other types of neutral salts, such as 1–2, 2–1, and 2–2 show mostly about the half values of the type of 1–1 (NaCl).

7. Pleolysis and the turbidity of the suspension of red cell nuclei. By WATARU NAKAMURA (*From the Department of Biochemistry, Yokohama University Medical School, Yokohama*). The suspension of nucleated red cells is most limpid at a certain zone of the concentration of neutral salt in aqueous solutions, and its limpidity diminishes gradually along with the decrease or the increase in the medium concentration (This medium concentration is termed as "pleolytic concentration"). The centrifuged sediments of hemolysates are resuspended in a new medium of corresponding concentrations. The saltsolution at pleolytic concentration now shows the maximum opacity, and the suspension tends gradually to be limpid with the decrease or the increase in salt concentration. For the estimation of the pleolytic concentration, this striking phenomenon offers a good criterion. But the resulting cells in these suspensions may undergo so serious alterations during the operations, that it can not be certainly said that the concentration showing the maximum turbidity intrinsically corresponds to the pleolytic concentration. By the experiments with the red colls of 34 species of fishes, the maximum turbidity of the red cell nuclei suspension was found at 0.20–0.25 M NaCl concentration, which is 4–5 times as high as the pleolytic concentration, indicating that the phenomena of the maximum turbidity and of the pleolysis are independent of each other.

Kamenosuke Shinohara: I wonder, the molar pleolytic concentration must be different when the various electrolytes, such as 1 : 1, 1 : 2, 2 : 2, are used. Furthermore, I think it better to represent the pleolysis as the function of the ionic strength. If so, it might be expected that the pleolytic concentrations would be found approximately at the same point in the diagram.

Wataru Nakamura: According to our results by representing the pleolysis as the function of ionic strenth, the pleolytic points of all the neutral salts in the disgram did not always coincide with each other. The experiments collaborated by Prof. Kuroda have proved that the pleolyic concentration is proportional to $(C_M \times Z_M)/r_M$; where C_M, Z_M and r_M represent the concentration, valenciy numbers and ionic radius of the cathion, respectively.

8. On the natures of the carbohydrates of normal human stomach and of the tissue of gastric cancer, both from persons belonging to blood group A. By HAJIME MASAMUNE, ZENSAKU YOSHIZAWA AND AZUMA MASUKAWA (*From the Medico-chemical Institute, Tohoku University, Sendai*). The carbohydrates were isolated from normal human gastric mucosa and gastric cancer tissues, by the modified Satoh's method. The preparations from the cancer tissues contained some mucosa carbohydrate as a contaminant when examined by electrophoresis. Both carbohydrates preparations resembles each other, but diverging only in the direct Osaki-Turumi reaction. By using paper chromatography, both preparations were proved to contain chondrosamine, glucosamine, galactose and L-fucose; they contained equal amounts of amino and non-amino sugars, but the cancer corbohydrate had far less iodine-values than the normal mucosa carbohydrate, suggesting that the cancer carbohydrate may be a higher polymer. Both preparations reduced about 3.2×10^{-3} moles HIO_4 per g., accompanying the disintergration of their non-amino sugars. The results by using the oxidant have indicated that both carbohydrates are con sisting of 3- or 4-carbon amino sugars and 2- or 4-carbon non-amino sugars, which are bound by glucoside linkage with the adjacent monosaccharide unit. The cancer carbohydrate preparation gave the cancer skin reaction (Matsubara) when applied in fresh state or after treating it with HIO_4. The cancer carbohydrate had specific anti-isohemo-agglutin, but its potency was much less than the normal mucosa carbohydrate; while both preparations exhibited no distinct difference with respect to anti-sheep-hemolysin activity against the anti-A immune serum. HIO_4 was shown to inactivate these serological activities of both carbohydrates.

9. On the mucin of submaxillary gland. I. By KOICHI TURUMI AND YOSHIHARU SAITO (*From the Department of Biochemistry, Fukushima Medical College, Fukushima*). The mucin prepared from bull submaxillary glands by Blix's method was autolyzed, and the autolyzate was fractionated with alcohol. There was isolated a substance having " direct positive color test " with *p*-dimethylaminobenzaldehyde, the positive color test for acetyl-hexosamine with *p*-dimethylaminobenzaldehyde (indirect procedure), the positive Sakaguchi's reaction and gave red color with Bial's orcinol test, but the other color tests for amino acids being negative. The substance was soluble in methano land water, and was found to contain glucosamine and arginine by paper partition chromatography after hydrolysis. The analysis of the substance was as follows: N 8.33%; glucosamine 6.77%; arginine 2.25%; acetyl 13.14%; ash 1.09%. From these results, it can be concluded that the substance is a complex compound composing of acetylglucosamine, arginine, and the 3rd unidentified component. The latter component is presumably a neuraminic acid-like substance from the point of view of its color tests.

10. Biochemical studies on 2-ketogulonic acid methylester. By KEISUKE YASHIMA (*From the Depertment of Medical Chemistry, Kurume Medical College, Kurume*). It has been demonstrated that 2-ketogulonic acid methylester shows antiscorbutic action, and that it is converted into vitamin C-like substance by the action of homogenates or water extracts of animal or plant tissues, or by SH-compounds. The production of this vitamin C-like substance is accelerated by Zn^{++}, Mg^{++} or veronal derivatives, but not by hydroquinone or vanillin, and rather inhibited by Cu^{++}, Ag^{+} or Hg^{++}. Total amount of vitamin C rises to the maximum after 5 hours and then decreases slowly. Among final products, the existence of 2,3-diketogulonic acid comes out positive; and its amount increases in due course of time. From these results, the following reaction steps are suggested: 2-ketogulonic acid methylester→ascorbic acid→dehydroascorbic acid→2,3-diketogulonic acid.

Shinohara Kamenosuke: If the vitamin C like substance in question is really vitamin C, the characteristic violet color must be once produced and disappear in a while in the presence of Fe^{+++}-salt is added to it. Have you ever tried this experimet?

Keisuke Yashima: Yes, I tried it. But, it seems that Fe reaction only comes out positive at a fairly sufficient concentration of ascorbic acid.

11. On the lipid in the immunsera. By HIROSHI CHINO (*From the Biochemical Institute, Matsumoto Medical College, Matsumoto*). During the immunization of rabbits with egg-albumin or sheep corpuscles, the contents of free-and conjugated-cholesterol, phospholipid, neutral fat and total fatty acid were found to increase in the immunserum. In order to know whether such increases of the serum lipids are essential to the formation of antibodies or not, rabbits were immunized separately with gelatin and with the erythrocytes of the rabbits of the same species. By fractionation of thus obtained immunsera with Na_2SO_4, the antibodies were entirely found only in the pseudoglobulin fraction, which was then proved to contain more lipid than that of normal rabbits. The immunological activity of pseudoglobulin fraction of the immunsera could not be eliminated by the treatment with ether, but it disappeared completely by digesting the fraction with trypsin or pepsin. In can be concluded that the increase in lipid content of the serum during the immunization is not essential to the formation of antibodies as well as to the antigen-antibody reaction; but the increase in serum lipid may rather be due to the stimulation by the injection of antigen substance.

12. Biochemical studies on the conjugated lipids. By TO IMAI, TOSHIAKI MURANAKA, TADAO SUGIMURA, MASAHIRO YAMAFUJI, SUSUMU KANEDA and TADASHI YAMASHIRO (*From the Department of Biochemistry, Hokkaido University School of Medicine, Sapporo*).

13. The enzymatic hydrolysis of cerebron. By YASUHIKO FUJINO (*From the Depertment of Medical Chemistry, Hokkaido University School of Medical, Sapporo*) By the action of the saline extracts of pig spleen activated with H_2S, cerebron was decomposed enzymatically, where galactose and the residue, possibly ceramide, were yielded. It has been demonstrated that the enzyme can split off the galactoside linkage, but not cleave the sphingosine-fatty acid linkage.

14. Studies on the conjugated lipids. VIII. The determination of the Hydrolyzable components of the conjugated Lipids. By TOSHIO SAKAGAMI, MANPEI YOSHIHARA and TADASHI SHIMOJO (*From the Department of Biochemistry, Sapporo Medical College, Sapporo*). Up to the present, the following components have been discovered as the hydrolysis products of the conjugated lipids from the animal tissues by several authors: glycerol, phosphoric acid, choline, ethanolamine, serine, inositol, hexoses, fatty acids, fatty aldehydes and sphingosine. Among these components the determinations of ethanolamine, serine, sugars and fatty aldehydes have not yet been popularized. Therefore, the methods for the exact determination of sphingosine and inositol have been devised. For the determination of fatty aldehydes (plasmals) Feulgen's method was modified in some points. The new method for sphingosine determination consists of hydrolyzing the lipids with methanolic HCl, extracting sphingosine with ether from the alkalinized hydrolysis mixture, oxidizing sphingosine with lead tetraacetate, isolating the fatty aldehyde as one of the oxidation products, and determining its concentration colorimetrically, successively. The improved method for the determination of sugars in the lipids is also established. The periodate method for the determination of ethanolamino and serine is modified and improved in some points.

15. Studies on the conjugated lipids. IX. The effects of toxic and dietatry fatty-liver factors upon the Composition of the tissue lipids. By TOSHIO TOKUSHIMA, MANPEI YOSHIHARA and TADASHI SHIMOJO (*From the Department of Biochemistry, Sapporo Medical College, Sapporo*). Using the previously reported methods, the authors measured the various hydrolyzable components of the conjugated lipids separately in the different tissues of the white rats suffered, from a varying grade of fatty liver by the following treatments: CCl_4 injection and then the continued administration of high fat-, high cholesterol- and raw liver-diets. It was recognized that the simple lipid fraction in liver increased exceedingly in all cases, while in other tissues it was comparatively constant, except in brains in which somewhat increase in was found cases of cholesterol- and raw liver-diets. In the conjugated lipid fraction, phosphoric acid, fatty acids, ethanolamine, serine, glycerol, sugars and sphingosine remained relatively constant in all tissues examined in every case, but choline decreased especially in liver in the cases of the cholesterol- and high fat-diets, and increased in brain by CCl_4 injection. Fatty aldehydes increased enormously in liver in the raw liver-diet.

16. Paper patition chromatography of lipid components. By HAJIME MASAMUNE and SEN'ITIRO HAKOMORI (*From the Medico-chemical Institute, Tohoku University, Sendai*). To identify the components of extremely complex lipids such as group lipoids, a stepwise chromatographic procedure has been devised. A lipid is hydrolyzed and the water-soluble and -insoluble fractions are subjected to separate processes as follows:

Chromatography of the Water-Soluble Fraction—First run: Solvent 1. *n*-Butanol saturated with water—phenol saturated with water—ethanol (5:3:1) (The run is effected in NH_3-atmosphere); Solvent 2. *n*-Butanol saturated with water-phenol saturated with water-glacial acetic acid (5:2:1). Solvent 3. *n*-Butanol saturated with water—phenol saturated with water ethanol (5:2:1.5). Different paper strips are used for the different solvents. Developers: Iodine (Brante), ninhydrin, aniline-phthalic acid, and lead acetate-H_2S. The developers are applied to each of the strips one after another in the order above beginning with iodine. In this way choline, ethanolamine and serine are identified and, besides them, amino-and non-amino sugars, amino acids, phosphoglycerol and inositolphosphate are detected, if present.

Second run: Chromatography ot amino acids in a usual manner.

Third run: Chromatography of sugars by Masamune and Maki's method—Hereby choline and ethanolamine are again identified.

Fourth run: Solvent 4. 80% Ethanol; Solvent 5. Ethanol-methanol-glycerol-water (2: 1: 1: 2); Solvent 6.80% Phenol-glacial acetic acid-water (1: 1: 1). Developer: Lead acetate-H_2S. In this case phosphoglycerol and inositolphosphate are distinguished.

Fifth run: Solvents 5 and 6, and 7 composed of glacial acetic acid, 80% phenol, *n*-butanol, glycerol, methanol and water in the volume ratios of 2.5: 2.0: 1 0: 0.5: 0.5: 10, are employed. Developer: Lead acetate-H_2S. The spot of the water-soluble fraction is irrigated through a $Ba(NO_3)_2$-band transversely drawn on the paper, whereby α-and β-phosphoglycerol are located at different hights.

Chromatography of Water-Insoluble Components—First run: Solvent 3. Three paper strips are used, and after the run one of them is treated with iodine, ninhydrin and methyl red consecutively, the second with Schiff's reagent and the third with alkaline permanganate. The presence of sphingosine, plasmals and higher fatty acids are thus proved. To find out higher fatty alcohols, a part of the fraction is acetylated and irrigated, and then both alkaline hydroxyalamine and $FeCl_3$ are applied in turn.

Second run: Individual plasmals, fatty acids and alcohols are identified here by reversed phase partition.

All the runs are ascending. An example with a crude protagon fraction was given.

17. On the methylation of pyridine in rabbit-organism. By YOSHIO OKUDA AND MASAMITSU KOBAYASHI (*From the Chemical Institute, Kobe University, Kobe*). In rabbit the power to methylate pyridine and to excrete ammonia are weaker than other animal species and phylyii. Where dose this difference in the detoxicating process between rabbit and other animals come from? To solve this problem a dose of 0.5 g pyridine was fed or injected to rabbits at intervals of a day over 7 months, and the amounts of methyl-pyridyl-ammonium-hydroxide exereted in the urine were determined. In most cases its exeretion increased gradually, perhaps by virtue of the adaptation in the long run. The simultaneous application of glycolic acid expedited the increase in methylated compounds of hyridine. This fact leads us to the conclusion that glycine is decomposed into ammonia and glycolic acid, and the latter may be playing a part in the formation of methylated pyridine.

Yukichi Hashimoto: According to my experience the aminal can methylate nicotiamide into N′-methylnicotinamide, which is, in turn, oxidized to N′methyl-6-pyridon-3 carboxylic amide. Have you carried out any experiments with pyridin-derivatives?

Masaji Tomita: The detoxication mechanisms of pyridine and its derivatives having side-chain are probably not the same. Our experiments are restricted to examine only the detoxication of pyridine itself having, of course, no side-chain. We have no experience upon the detoxication of nicotineamide.

Ryoji Ito: How do you think of the fact that N-methylpyridine will be further oxidized at α-position?

Masaji Tomita: We have never obserrved the α-oxidation of N-methyl compounds of pyridine, quinoline and isoquinoline.

18. Influence of glucose on the fat content of the partial hepatectomized rat. By YOSHIHARU UMEMURA (*From the Department of Biochemistry, School of Medicine, Keio University, Tokyo*). When a portion of the rat liver was excised and the animal left hunger for 36 hours, the far content increased remarkably in the remaining portion of the liver. To such animals, glucose solution (2.5 ml. of 50% solution) was given, and after 4 to 6 hours, the effect was observed with the following results: (a) In the rats, 10 hours after partial hepatectomy the increase in the fat due to the excision of the liver was suppressed; whereas liver glycogen increased; (b) At the stage of 24 hours after the hepatectomy the action of glucose suppressing liver fats was weak, and the increase in the glycogen was small; (c) With the rats which hab the lapes of 36 hours, the suppressing action of glucose was not observable, and the glycogen did not increased. These results may clearify that the action of glucose suppressing the increase in liver fat appears for a short time after the increase in the fat, but it does not appear after the lapse of longer time.

19. On the dehydrobromination of 3,12-dihydroxy 7-ketocholanic acid. By TATSUMI BABA ADN KANZO SASAKI (*From the Department of Biochemistry, Hiroshima Medical College, Hiroshima*). Formerly, bile acids of the allo-series were transformed from 3-keto acid as bromo derivatives, but this experiment was undertaken with the idea that a simpler transformation would take place if 7-keto acid was used. However. at C_3 the bromo derivative of 7-keto acid having a hydroxyl group is very unstable and readily decomposed. This is considered to be due to the stereochemical contiguity of the 3-hydroxyl group and the 6-bromine atom. In this manner, through the acetylation of the 3-hydroxyl group, a stable bromo derivative, ethyl 3-acetoxy-6-bromo. 7-keto·12-hydroxycholanate was successfully gained. Through its dehydro bromination with silver nitrate in pyridine, $\Delta^{3,5}$-7-keto·12-hydroxycholadienic acid (m.p. 205°) was procured (the bromine atom is probably β-oriented, since the bromo derivative is very resistant to dehydrohalogenation) and by its partial catalytic hydrogenation with hydrogen and palladium catalyst, Δ-^{5}-7-keto·12-hydroxy-cholenic acid (m.p. 204°) was obtained. Its absorption spectrum shows the existence of α, β-unsaturated ketone. Namely, it shows a strong absorption band in the region of 238mμ and the second band of low intensity with a maximum at 320mμ in alcohol. This concides with the product gained through dehydrobromination of the bromo derivative of 7-keto·12-hydroxycholanic acid. 7,12-Dihydroxyallocholanic acid (m.p. 199°) was successfully gained from Δ^5-7-keto·12-hydroxycholenic acid through catalytic hydrogenation with hydrogen and platinum catalyst.

20. Dehydration of hyodesoxycholic acid. II. A synthetic pathway of bile acids to progesteron. By KAZUMI YAMASAKI and ISAMU USHIZAWA (*From the Department of Biochemistry, Medical School, Tottori University, Yonago*). The hydroxycholenic aciso derived from hyodesoxycholic acid was reduced (catlytically) to β-3-hydroxycholanic acid, which testified the previous report. Diphenyl carbinol of hyodesoxycholic acid was dehydrated with phosphorus oxychloride, followed by dehalogenation. By this way the very β-3-hydroxybisnorcholenyl diphenyl ethyenen was prepared, which was derived from the above hydroxycholenic acid.

Junichi Kawanami: Will you kindly explain the following points? (1) Condition for dehydration of hyodesoxycholate. (2) The melting point of methyl hyodesoxycholate. (3) Melting points of some compounds you derived.

Kazumi Yamasaki: (1) Methyl hyodesoxycholate in pyridine is heated for 3 hours on water-bath. (2) The melting point of methyl hyodesoxycholate used is 95°. (3) The melting points are 100–101° for methyl 3-chlorocholenate, 155–156° for methy 3-acetoxycholenate, 135–136° for 3-chlorobisnorcholenyldiphenyl ethylene, 168–169° for β-3-acetoxybisnorcholenyl diphenyl ethylene and 174–175° for 3-hydroxybisnorcholenyl diphenylethylene.

21. On the bile acid in the bile of *Rand nigromaculata nigromaculata* and *Misgurnus anguillicaudatus*. By TOSHIO KOMATSUBARA and HIDEYUKI NAKAMURA (*From the Department of Biochemistry, Hiroshima Medical College, Hiroshima*). From the bile of *Rana nigromaculate nigromaculate*, α-trihydroxydisnorsterocholanic acid ($C_{26}H_{44}O_5$, m.p. 172°) and β-trihydroxybisnorsterocholanic acid ($C_{26}H_{44}O_5$, m.p. 196°) were isolated. They were proved to exist partly as free acids and the greater part as conjugatedbile acids with taurine. This is the first time that these acids have ever existed as conjugate forms with amino acid in stero bile acid. From the bile of *Misgurnus anguillicaudatus*, a new neutral bile acid, conjugated with sulfuric acid ($C_{30}H_{53}O_5SO_4Na$, m.p. 192°) was isolated. From this, hexahydroxysteroid ($C_{30}H_{54}O_6$, m.p. 242–3°) was isolated through hydorlysis. In order to decide the chemical structure of the side-chain of the stero bile acid the following synthesis was attempted. Using cholic acid as start material, normaltrihydroxynorsterocholanic and ($C_{27}H_{46}O_3$, m.p. 201°) was synthesized by means of diazoketone synthesis and malonester synthesis. This is the first time that the synthesis of stero bile acid was successfully accomplished.

22. Urinary excretion of dehydrocholate administered under various conditions. III. By KAZUMI YAMASAKI and NAOYUKI MIYASHITA (*From the Department of Biochenistry, Medical School, Tottori University, Yonago*). The urinary excretion of dehydrocholate after its adminstration to rabbits makedly increasesd when the animals hade been intoxicated with CCl_4. But, the excretion was rather reduced to the normal level by the simultaneous adminstration of vitamin B_2 or methionine together with dehydrocholate. The implantation of ox pituitary gland and the ligation of the ductus choledocus resulted in a difinite increase in the urinary excretion of dehydrocholate. The increase in urinary excretion of dehydrocholate caused by CCl_4 was quite different from that encountered by the ligation of the ductus choledocus. These facts may suggest the site of biosynthesis of bile acids in the light of pathological knowledges.

23. Studies on bile acids of bull frog and toad, and the micro-biological degradation of bile acids. By SHOHEI HAYAKAWA and MASAKIYO KURODA (*From the Department of Biological Chemistry, Okayama University Medical School, Okayama*). From the bile of bullfrog, three sulfate esters of m.p. 161°, 188°, and 198° were isolated. By hydrolyzing them unsaturated compounds were obtained: from the ester of m.p. 161°, two substances of m.p. 177° and 196°; from the ester of m.p. 188, a substance of m.p. 177°; and from the ester of 198°, two substances of m.p. 238° and 177°. These last two substances (m.p., 238° and 177°) are considered to be the isomers with epimers of hydroxyl radicals, since both give the same oxidation product of m.p. 240°. A new unsaturated bile acid (m.p., 176–9°) was isolated from toad bile, having a formular composition of $C_{27}H_{44}O_5$ with a double bond in its side chain; its methyl ester of m.p. 184–6°; its hydrogenated acid of m.p. 169–172°; and its hydrogenated keto acid, of m.p. 232–4° were obtained. Cholic, glycocholic, dehydrocholic, desoxycholic, homocholic and cholanic acids were found to serve as a good C source in the culture medium of *Actinomyces*. From the medium containg cholic acid, two acids were isolated as the degradation products: the one had molecular weight of 400–412°, decomposed at 280–2°; and the other decomposed at 248° (sinter 230°). The former, presumably a monocarboxylic acid, is decomposed by caustic alkali, giving a colored material, and the acid is considered to be a primary oxidative degradation product of cholic acid.

24. On the degradation products of ketonic bile acid in the dog. By KYUTARO SHIMIZU (*From the Department of Biological Chemistry, Okayama University Medical School, Okayama*). The liver of the dog was perfused with dehydrocholic acid, and the excreted urine and bile juice were analyzed. From the urine, cholic acid, dehydrocholic acid and a neutral substance (f.p., 232–250°) were isolated. The latter neutral substance had the composition of $C_{19}H_{28}O_4$, being presumably a etiocholane derivative; its oxime decomposed at 238°. From the urine dehydrocholic acid and a neutral substance (f.p., 239°) were obtained. This neutral substance had the composition of $C_{21}H_{32}O_4$, being likely to be a pregnane derivative.

25. Studies on iron absorption II. By YOSHIO YOSHINO (*From the Nutritional Laboratory, Nippon Medical College, Tokyo*). A strip of the small intestine (about 50 cm. length) of adult rabbit was perfused with Tyrode solution, containing 0.2% $FeSO_2$ or $FeCl_2$. The autoxidation of iron inside the intestine during the perfusion gave no influence upon the iron absorption. The absorption proceeded equally with both $FeSO_4$ and $FeCl_2$, and was decreasing with time. When the perfusing solution was saturated with O_2 by different ways: one saturated by suspending red cells, and the other by simple gassing, the iron absorption is inferior in the latter case. With the intestine containing $FeSO_4$ at a concentration of 0.2% the iron absorption is reduced more pormptly by the perfusion with Tyrode solution containing 0.025% NaH_2PO_4 than the control. The total amount of ferritin in the extract of the intestin mucosa after absorption of iron was found to be far greater than that of the unabsorbed mucosa. The iron absorption was also accelerated by perfusing serum diluted with Tyrode solution.

26. Studies on non-hemin iron. BY YOSHIMASA YONEYAMA AND KUNIO KONNO (*From the Department of Biochemistry, School of Medicine, Tokyo University, Tokyo*). Asuspension of minced tissue in physiological saline solution was heated at 80° for 10 minutes, and centrifuged (the obtained precipitates are named, PI). The filtrate was heated again at 100° for 30 minutes and centrifuged (PII). Acetate buffer solution was added to the filtrate, its pH was adjusted to 4, the solution was heated at 100° for 30 minutes, and separated into precipitates (PIII) and supernatant fluid (PIV) by centrifugation. The contents of nonhemin iron were determined with all these fractions (PI, PII, PIII, PIV) by Bruckmann and Zondek's method. The rats were killed after the oral administration of radioactive iron isotope, and the specific activities of 4 fractions (PI, PII, PIII, PIV) of liver, spleen and kidney were measured by the method of Voshurgh, Flexner and Cowie. The results were as follows: (a) PI fraction contained hemosiderin most possibly; in spleen the greatest amount of iron, was contained in PI (predominantly hemosiderin); the rate of iron incoporation into PI was the slowest in liver; (b) Iron in PII fraction was mainly consisting of ferritin iron; in liver, PII was very rich in iron, and the incorporation rate was also very high; the immunological experiments with ferritin in the dog indicated that the iron in PII was truly ferritin iron; (c) PIII fraction was considered to contain the iron bound with nucleoprotein and other high-molecular compounds which were not precipitated with PI and PII, but the iron content in PIII was very small. (d) PIV fraction was not such high-molecular compounds, and there was little or no iron in PIV. Among the organs tested, spleen was found to be the richest in non-hemin iron.

27. Studies on the alternation phenomena of catalatic and peroxidatic activities of metal catalysts as copper, hemin and hemoglobin. BY TARO TOMIMURA AND KEIZO TSUSHIMA (*From the Biochemical Laboratory, Nippon Medical College, Tokyo*). The velocity of peroxidatic decomposition of ascorbic acid with H_2O_2 catalyzed by copper ions were determined manometrically in N_2-atmosphere. The addition of pyridine accelerated the peroxidatic decomposition of ascorbic acid. When the ratio of the concentration of ascorbic acid to that of H_2O_2 was 2: 1, the catalatic action of copper ions was completely suppressed. Similar results were obtained by using pyridine-hemin or methemoblobin prepared from catalase-free blood, as a catalyst. The observed suppressing effect was thus a non specific one. A prefect suppression of catalatic activity was observed at the concentration ratio of ascorbic acid to $H_2O_2=1:1$. The catalatic activity of copper ions was markedly accelerated by the addition of ascorbic acid in the initial stage of the reaction, and thereafter it turned to decrease rapidly. Similar effects were found by use of pyrogallol or hydroquinone as a substrate; the acceleration of the catalatic and peroxidatic activities under this condition may be correlated to the grade of $Cu^{++} \rightleftarrows Cu^{+}$ turnover.

28. On the mechanism of the reaction of the choleglobin formation with special reference to the role of electric charge of hemoglobin. BY GORO KIKUCHI AND TAKASHI OGAWA (*From the Biochemical Laboratory, Nippon Medical College, Tokyo*). Besides choleglobin, methemoglobin was formed simultaneously in the reaction system consisting of hemoglobin, ascorbic acid and oxygen. In this experiments, oxyhemoglobin was prepared from catalase-free blood (*Anenzymia catalatica*), and methemoblobin was further prepared from oxyhemoglobin. The reaction of the choleglobin formation proceeded far slowly in the initial stage when oxyhemoblobin was replaced by methemoglobin than in case of oxyhemoglobin as the starting material; in the later stage, the reaction proceeded at the same rate as in case of oxyhemoglobin. The amount of decomposed ascorbic acid determined chemically was rather higher even at the beginning stage of the reaction. These facts suggest that the formation of choleglobin takes place only after methemoglobin was reduced to hemoglobin by ascorbic acid. Once the choleglobin formation reached its steady stage the the rate of choleglobin formation and that of ascorbic acid oxidation run parallel with each other. The inhibitory effect of catalase upon the reaction of the choleglobin formation was distinct also at the initial stage. The reaction rate, especially the rate of choleglobin formation was influenced greatly by temperature. It can be assumed that some intermediate compounds of a relatively high peroxidatic activity for the oxidation of ascorbic acid may be formed during the conversion of hemoglobin into choleglobin.

Kamenosuke Shinohara: Is there any direct evidence for the production of H_2O_2 during the reaction? If not, the following method would be advisable. After the ascorbic acid remaining in the reaction mixture has been oxidized by I_2 KI and molybdate are added to the solution in slightly acidic medium, and the freed I_2 can be then measured by iodometry.

Goro Kikuchi: We have not been able to confirm directly the production of H_2O_2 in this reaction system. However, we know several facts in favor of our assumption. For instances, (1) it is well known that H_2O_2 is produced when ascorbic acid is oxidized by oxygen in the presence of some catalyst such as Cu^{++}; (2) "630-intermediate compound" is formed in the reaction system of pyridine-hemin, ascorbic acid, and H_2O_2 just as same as in the system of pyridie-hemin, ascorbic acid and O_2; (3) choleglobin is obtained also by the direct addition of H_2O_2 to O_2Hb in the absence of ascorbic acid; and (4) choleglobin formation is inhibited profoundly by catalase. Willingly we will try the method suggested by you.

29. On the processes of bile pigment formation *in vitro*. BY KENJI YAMAOKA AND KIYOWO KOSAKA (*From the First Medicine Clinic, Okayama*

University Medical School, Okayama). By the action of ascorbic acid and O_2 on hemoglobin, the reaction proceeded in several stages. The intermediate compounds in each reaction stage from hemoglobin to bile pigment were separated by chromatgraphical analyses. The "easily splitt off blood iron" (G. Barkan) quite well corresponded to verdohemoglobin (M. Engel). This verdohemoglobin was considered to be a representative material for the intermediate compounds in the bile pigment forming reaction. In all reaction stages, freed iron was fairly well changed to ferric state; and the "easily splitt off iron" became ferric when verdohemoglobin formation reached the maximum. These facts indicate that the iron atom in verdohemoglobin was possibly in ferric state. The free iron was present in the reaction mixture from the beginning stage, but the iron to be liberated from verdohemoglobin increased abruptly only after verdohemoglobin began to decrease.

Goro Kikuchi: According to the experiments of Yamada, the amounts of the formed choleglobin and the amounts of the liberated iron by 0.4% HCl or trichloracetic acid have been confirmed to be almost completely parallel with each other. I wonder, therefore, it is rather of less significance to distinguish the iron into "easily splitt off blood iron" of Barkan and so-called "freed iron" in your experiments.

Kenji Yamaoka and Kiyowo Kosaka: After the method of estimation for "easily split off blood iron" originally found by G. Barkan, we have carried out the estimation of the changes of so-called "easily split off blood iron" contained in those extremity circulations, on such occasions as hemolytic jaundice patients or paroxysmal hemoglobinuria patients in their hemolytic attacks, or patients of malaria at its fever-attack, or when we have administered either hemoglobin, phenylhydrazine or water to water to healthy animals (rabbits, dogs); at the same time we examined the changes occurring in serum bilirubin. Results were that, even prior to the increase of bilirubin, for once-for-all, remarked an increase in so-called "easily split off blood iron"; while no increease in serum iron). Such a clinical results of animal test led us to conclude what is indicated as "blood iron" above mentioned, might not be the very intermediate product in the course from blood pigment to bilirubin. Therefore, this *in vitro* experiment is nothing but a test conducted in order to clarfy this point. So, in that sense, the "blood iron" has been laid under our notice. Also, because of these facts, namely, on account of the absence of any difference between the frec iron and "easily split off blood iron," your opinion may be concluded as a miss.

Goro Kikuchi: I would like to ask you, if the ferric iron was proved by use of reducing agents, such as ascorbic acid and hydroxylamine.

Kenji Yamaoka and Kiyowo Kosaka: It means, because of indomitable appearance of ferric ion the transformation into ferrousiron by means of ascorbic acid or HCl has not taken place as yet, that would be iron which was able to be vindicated, in the form of ferric ion. The verdohemoglobic stage as has been pointed out during this experiment, is a synthetic one for several different stages, and could be determined as ferric form, at least in those stages where iron could be easily separated.

30. On the chromatographic ana-

lyses of bilirubin-azo-pigment. By KENJI YAMAOKA and KIYOWO KOSAKA (*From the First Medicine Clinic, Okayama University Medical School, Okayama*). Bilirubin-azo-dye has been prepared by the action of alcohol and diazo reagents on "indirect bilirubin," which can be separated in one layer by use of a chromatogramm filled with active alumina and developed with alcohol. But the position of the layer varies with the kind of alcohol used for developing. With the dye prepared from the natural ester type of bilirubin the separation of the bilirubin-azo-dye from "indirect bilirubin" can be performed chromatographically by using ethanol, but not by methanol as beveloping solvent. Such separable proberties of both dyes may possibly be due to the ester bond ot propionic acid of a side chain of bilirubin. It can be assumed that alcohol plays a rôle in conversion of "indirect bilirubin" into the direct one by forming an ester bond and that the natural type bilirubin possesses a methyl ester.

31. On the urobilinogen in urine. By HISAE URANO (*Form the Department of Biochemistry, School of Medicine, Gunma University, Maebashi*). Ehrlich's reaction for urobilinogen in urine is often positive (intense red color) without heating procedure in cases of normal urine. In order to evaluate the reliability of the Ehrlich's reaction as a liver function test, the reaction is carried out as follows: Urine is collected at intervals of 30 minutes or one hour. The urine is diluted to 200 and 400 ml, respectively. With this diluted urine the reaction is conducted as in the original method. With normal urine red color does not appear; but if the rate of urobilinogen excretion is above normal, moderately intense red color now appears. In the case of the highest rate of excretion the amount of urhbilinogen is 0.4 mg./hour; normal value is 0.05–0.20 mg./hour; and the reaction is negative with the urine containing less than 0.1 mg. urobilinogen per hour. The rate of urobilinogen excretion is found to be generally higher in the morning and the daytime than in the evening and at night; but the maximum rate is not necessarily found at 2–4 o'clock P.M. Diuretic drugs markedly stimulate the excretion of urobilinogen.

Osamu Nishikaze: How do you think about the reason of diurnal variation of the excretion of urobilinogen?

Hisae Urano: Some of the physiological functions show a rhythmical change, being high by day and low by night. This may be considered to have a connection with the questioned phenomenon, but its detailed mechanism is net yet known.

32. Studies on the effective substance of bone marrow against dystrophy. I. The protective action of bone marrow extracts against the starvation of mice. By KAICHIRO KURODA, JUN NAKATANI and SHIGEKO UMEDA (*From the Department of Biochemistry, School of Medicine, Tokushima University, Tokushima*). In order to ascertain the effective fraction of beef bone marrow extracts against dystrophy, the mice were fed on the behalved basal diet to force them into the dystrophic state. The average life of the mice was the longest when 0.1 g. precipitated fraction at pH 4.7 (acetic acid) or 0.1 g. supernatant fraction of the bone marrow extracts was added to 1.5 g. raw wheat flour (basl diet behalved); it was moderate with the mice fed on 0.1 g. purified gelatin or on

0.05 g. fat soluble fraction of the bone marrow soup; and it was the shortest with the control. The body weight decreased also at the same rate in all cases. Thus the effective substance was found to be contained in the water soluble fraction of bone marrow. Just before the death from hunger, the benzidin test of jeces became positive and ulcer was observed in stomach and small intestine in 80% of the starved mice.

33. Studies on the action of tetraethyl lead. I. On the blood levels of sugar, lactic acid and iodate values in tetraethy lead poisoned rabbits. By HANAKO YOKOI and KIKO GOTO (*From the Biochemical Laboratory, Nagoya City University Medical School, Nagoya*). From rabbits each 10 ml. of blood was taken 3 times every 2 hours and at the 24th and 48th hour after the administration of 63 g./dl Pb $(C_2H_5)_4$ in gasolin 0.6 ml.kg. body weight) by subcutaneous injection. The control rabbits showed the following blood values: sugar (Hagedorn-Jensen) 0.104 g./dl.; lactic acid (Hirsch-Kaufmann) 21.58 mg./dl; iodate value (Nishigaki) 0.172 ml. of $N/200$ KIO_3 per 0.5 ml. By the administration of Pb-$(H_2H_5)_4$, the blood sugar decreased slowly after the injection, turned to the normal level after 48 hours. The lactic acid began to increase soon after the injection, reached the maximum value (201.4 mg./dl.) at 4th hour, and turned to decrease slowly to the normal level at 48th hour. The iodate value markedly decreased soon after the injection, and increased so slowly that it did not regain its normal value even after 48 hours.

34. Studies on the action of tetraethyl lead. II. On the various phosphates and phosphatase activity of the central nevous system of tetraethyl lead poisoned rabbits. By HANAKO YOKOI and KIKO GOTO. (*From the Biochemical Laboratory, Nagoya University Medical School, Nagoya*). Rabbits were received daily 0.06 ml. of 64 g./dl Pb $(C_2H_5)_4$ in gasolin by subcutaneous injection. After about 2 weeks they were sacrificed by air embolus. The central nervous system was divided into cerebrum and cerebellum (pons and medulla oblongata inclusive). Tissues were homogenized with a chloroform-water mixture, and autolyzed for 24 hours in an incubator. The filtrate was used as the enzyme solution. There was no distinct difference in both the amounts of total P and acid-insoluble P between cerebrum and cerebellum. Residual P decreased, but pyrophosphoric acid, hexosephosphoric acid, inorganic phosphoric acid, acid-soluble P and lipid P rather increased. The optimum pH of β-glycerophosphatase action was found at 4.7 and 9.6, at which the activity was, in general, lower than those of the normal origin.

35. On the carbohydrate in casein. I. By MASAHIRO MAKI (*From the Medica-chemical Institute, Hircsaki University, Hirosaki*). Two biuret-negative specimens of the carbohydrates in casein were prepared with and without tryptic digestion, respectively. They were different somewhat in solubility but resembled well in other respects. They showed neither the direct nor the indirect Osaki-Turumi's reaction. Paper partition chromatography revealed glucosamine, galactose and mannose as sugar components; and aspartic acid, glutamic acid, glycine, alanine, either proline or hydroxyproline, either methionine or valine and either leucine or isoleucine as amino acid components. Analyses of undigested and trypsin digested carbohydrate preparations: N 2.0 and 2.1% glucosamind 12.9 and 12.2%; non-amino sugars estimated by indole method 59.8 and 56.7% by orcinol method 57.4 and 56.3%, and diphenylamine method, 61.3 and 53.8% (as an equimolecular mixture of mannose and galactose); the molecular ratio of hexosamine to total hexoses 1 : 4.6 and 1 : 4.6; acetyl 0.0 and 0.0%; iodine consumed as glucose 4.3 and 4.0%; molecular weight calculated from iodine values, 4130 and 4500; ash, 0.0 and 0.1%; $[\alpha]^D$, −56.5° (8°) and −54.8° (7°), respectively. One of the specimens was examined by electrophoresis, but it is demonstrated only one boundary.

36. The effect of substances related to the C_4-dicarboxylic acid cycle upon ketosis. BY MASAHARU NIWA (*From the Department of Medical Chemistry, the 2nd Tokyo National Hospital, Tokyo*). The effects of substances related chiefly to the C_4-dicarboxylic acid cycle upon the formation of keton bodies caused by starvation or CCl_4-intoxication were studied. Succinic acid, fumaric acid, malic acid and glucose decreased the total ketone bodies in blood (determined by Greenberg and Lester's method) to an equal extent, in proportion to the original amount of blood ketone bodies. The similar results were obtained by glutamic and aspartic acids; L-aspartic acid decreased the total blood ketone bodies about 2 times as much as DL-aspartic acid. There was a significant correlation between total blood ketone bodies and total fats in liver (Gragdon's method) with a correlation coefficient (r) of 0.76, the portions of which were increased by hunger beyond normal grade.

37. Azotometric determination of choline. BY HIKOO MATSUMURA (*Form the Department of Medical Chemistry, Keio University, Tokyo*).

38. Relation between alarm reaction and Donaggio's reaction of urine. II. BY HIROSHI SEKIMOTO, YOSHIO UTSUNO, MAKOTO YONEZU AND HISAE URANO (*From the Department of Biochemistry, School of Medicine, Gunma University, Maebashi*). The effect of various stressing agents on human and albino rats were studied in respects to the grade of alrm reaction and of the Donaggio's reaction. All stresses including cold, burns, fracture, muscular exercise, administration of vaccine, ACTH and cortisone, caused an elevation of DRY (Donaggio's reaction modified by Yamazoe) for several days or longer in parallel with the changes in urinary total N, but DRY did not change so remarkably when human or animals were fed on high protein diet. By assuming that DRY may be an index of protein catabolism, the DRY seems to be affected rather by "endogenous" protein metabolism, while the urinary total N changes with the amount of protein intake ("exogeneous" protein metabolism). DRY is considered to be a better index of alarm reaction than the ratio of uric acid to creatinine.

Osamu Nishikaze: How do you think about that the elevation of Donaggio's reaction occurred in relatively later stage (reconvalescence period) in your data of burns?

Saburo Yamazoye: It seems us that the burns as stress are so slight, that we are not sure whether the elevation of DRY is the direct effect of burns or the secondary effect of infection or toxic substances. The burns were of the 2nd degree caused by a heated brass disk of 2 cm. diameter in two places on the back of the rat.

39. On the operative stress and the adptation response. By MORIO MURASE, TATSUTO SHIMIZU, MASANORI KUMADA, NOBUO SUZUKI, SUSUMU HASEGAWA and NOBUKO KATO (*From the Ogawa Laboratory, The Research Institute of Enviromental Medicine, Nagoya University, Nagoya*). The adaptation response to the various environmental changes and stresses including fatigue was studied from the viepoint of the pituitary-adrenal system; especially on the metabolic changes after surgical operations. After fatigue or operative stresses, the total reducing substances in urine (total reducing power: TR) increased rapidly and remarkably. These substances were analyzed into some components such as uric acid (U), creatinine (C), genuine sugar (G) *etc.* by authors' ferricyanide method, and these values were represented in terms of glucose equivalent. When TR increased, G also increased, playing the leading part in the increment of TR. Comparing the ratio of G to TR (G/TR) with some other ratios, such as Thorn's U/C, Nishikaze's O/K, Muller's O/N, it is obvious that G/TR increases more rapidly and remarkably than other ratios. GTR can be very simply and accurately determined by the ferricyanide method. Thus the ratio, G/TR, is preferably available for an index of the adaptation response. Ogawa's colloid reaction for the practical index of protein-catabolism in cases of the sugical operation stress showed that G/TR and Ogawa's colloid reaction were suitable indexes for adaptation response. After thoracoplastic, albumin decreased initially and then increased to or over preoperative levels; fibrinogen increased remarkably; but α-globulin decreased temporarily. α-and β-globulins changed rather variably.

40. Biological defence reaction and Vakat oxygent. By OSAMU NISHIKAZE (*From the Institute for Tuberculosis, Hokkaido University, Sapporo*).

41. The disturbance of the liver enzyme system in the burned rabbits. By TAKAAKI INOUE, YASUHIKO BABA and TORAO KAWACHI (*From the Mitsui Medical Research Institute for Inatsuki, Inatuki-machi, Fukuoka-ken*). The effect of burns on purine metabolism in rabbits was studied. The deproteinized solution of the normal rabbit serum showed the spectral end-absorption due to allantoin. The same spectrum was generally observed with the deproteinized solution of the burned rabbit serum; but only in the burn on the abdomen a characteristic absorption band at 270 mμ was recognized 2 days after the burns. Such a selective absorption spectrum seems to be due to purine derivatives, probably in chief to xanthine. The occurrence of the other purine derivatives except allantoin in rabbits proves the disorder of purine metabolism in the severe burns on the rabbit abdomen. From the fact that xanthine is found in the deproteininzed serum, it can be presumed that the purine oxidation in liver catalyzed by xanthine oxidase may be disturbed as a result of the abdominal burns, since xanthine oxidase is not contained in the rabbit kidney.

24. Studies on drug-diabetes. By GORO KUSUNOKI, SEIICHIRO YAMASAKI, FUSAKU ASUKA, TO-

MOO MIHARA, YUKIMASA HIRATA, SEIICHI SANO, YO INADA AND SYUNSUKE FUJII (*From the 2nd Clinic of Internal Medicine, Kyushu University, Fukuoka*).

43. Studies on diabetes mellitus induced by the administration of quinoline derivatives. BY KOZO OKAMOTO, TADAYOSHI FUJIWARA, KENZO SUKENARI, KINICHIRO FUKUTOME ((*From the Department of Pathology, Kobe Medical College, Kobe*), TSUNEO MATSUO AND AKIHIKO MUSASHI (*From Kobe Womens' College of Pharmacy, Hyogo-hen*). The diabetogenic or diabetes-preventing action of some toxic substances having affinity to Zn was studied. Among 20 quinoline derivatives, the following compounds were diabetogenic to rabbits: oxine, 8-methoxyquinoline, 2,4-dimethyl-8-hydroxy-quino line, 5-(phenylazo)-8-hydroxyquinoline, 5-(*p*- or *p*-tolylazo)-8-hydroxy quinoline, 8-hydroxyquinalidine, 5-(phenylazo)-8-hydroxyquinalidine, 5-(*p*-hydroxy-phenylazo)-8-hydroxyquinalidine. The diabetestes-preventing action was rather observed with quinalidinic acid, 5-nitroquinalidini acid, xanthurenic acid, and atophane. In cases of diabetes mellitus of rabbits induced by the above diabetogenic compounds, the changes in blood sugar, and histochemical features of Langerhans'-islets were quite the same as those in cases of alloxane or oxine diabetes.

Yahito Kotake: I could surely prove the occurrence of diabetic symptoms by xanthurenic acid with many examples using white rat. To examine the effect of xanthurenic acid upon animal body (the effect of occurring diabetic symptoms), I think it necessary to examine first the natural compound isolated from urine, and then the synthetic one, using the natural one as a standard. I think it especially necessary in synthetic xanthurenic acid because it is often impure. It is dangerous to use immediately the synthetic compound like you did, the melting point of which is lower than the natural one. I used white rat, and the difference in animals must be considered, but I think that this effect surely exists also in rabbit, even though the effect is weaker than in white rat by the difference in the effect of xanthuric acid-decomposing enzyme in liver.

Kozo Okamoto: What I have reported now is concerning the results from our experiments with rabbits and white rats on a synthetic xanthurenic acid (m.p. 186° (decomp.) in winter season. If there be some differents between synthetic xanthurenic acid and the natural one, we wish to continue the experiment with the natural product.

Seiichiro Yamasaki: Alloxan, one of diabetes-preventing substances, is known to react with SH-group. Thiolactic acid can prevent also alloxan-diabetes. I think that the initial hyperglycemia is of hepatogenic nature, because blood K value in alloxan-diabetes runs in parallel with blood sugar value; if it should be of adrenal orgin, the adrenal must be stimulated to secrete adrenalin, and subsequently blood K value must be diminished. I think, therefore, that the hyperglycemia by Tb-1 is hepatoginic one, since, in this case, glucose tolerance test gives lower fasting sugar level, the peak at about 200 mg.% sugar concentration and a faster decrease to the normal level.

Kozo Okamoto: (1) As to the mechanism of the preven tion of alloxan diabetes, I think that it is of both pancreatic and extrapancreatic origins. To day I have explained briefly about the pancreatic prevention, that is, only on the method of diabetes prevention by blockading of the islets. But, on the other hand, we have been studying steadily on the extra-pancreatic prevention, and we have reported on it at the Japanese Pathological Society (Nov. 1951 and Apr. 1952). (2) As to the cause of the appearance of the initial hyperglycemia, I have not reached any definite conclusion as yet. (3) As to TbI I have been unable to discern any change in the islets of Langerhans on examining the rabbits which died through administering a large dose of TbI.

44. Studies on cyanide. XII. Biochemical investigations of Yoshida-sarcoma and surviving process of tumor tissues. By YOSHIFUMI OTAGIRI, SINITI SUMITOMO, SIGEO HOKARI and KIYOSI SASAMOTO. (*From the Pharmacological Department, Nippon University Medical, School, Tokyo*). The cells of Yoshida-sarcoma were transplanted to rats intraperitoneally, and the autotrophic sarcoma cells, ascites fluid and urine were used for experiments. NH_2CN-reaction came out positive with sarcoma ascites fluid though the reaction was negative with usual ascites fluid. The ascites fluid of sarcoma showed stronger Sakaguchi's reaction than the control. Rhodanase was scarcely distributed in spleen and kidney which were rarely affected by the occurrence of primary or metastatic sarcoma. The excreted urine of sarcoma rats showed high rhodanase activity. It is considered that the amino acids required for the formation of tissue protein during the growth of Yoshida sarcoma may be introduced in part by the transamination of NH_2CN.

45. Comparative studies on sero-diagnostic reactions of cancer. By KIMIKO KATAYAMA, TETSUO HAYASHI, MIEKO YAMADA (*From the Biochemistry Department, the National Institute of Public Health, Tokyo*), KIYOHITO OKUBO and GENSUKE ISHIKAWA (*From the Branch Hospital of Tokyo University, Tokyo*). To study the chemical and enzymatic changes in plasma of some cancer patients, the contents of -SH and -S-S- groups of plasma protein were determined by amperometric titration. It has been found that -SH groups as well as -S-S- groups are clearly diminished in the plasma of patients with cancer of digestive tract as compared with those in normal individuals as well as the patients of tuberculosis and pregnant women. These results may be attributed partially to the decrease in plasma albumin and partially to the change of the nature of plasma. It has been further confirmed that the plasma anti-tryptic activity of gastric and uterine cancer patients show considerable increase comparing with those of the normals and non-malignat tumor patients. To elucidate the genesis, the plasmin released by chloroform treatments was measured; but there was no difference in plasmin between cancer patients and the normals. With gastric and uterine cancer as well as non-cancer patients or normal individuals, the serum phosphatase assay in the presence and absence of Co ions did not indicate the specific tendency to the cancer patients. Furthermore, the deficient heat coagulation tests were excellent in the diagnosis of cancer, especially of the gastric cancer.

Kohei Hayashi:—I think it necessary to consider the fact that fibrinolysin can not completely disintergrate protein into amino acids, but it yields probably only peptides.

Kimiko Katayama: We measured the phenol bodies released during the incubation after treatment with trichloracetic acid. But our examinations could not exclude the released peptides containing phenol bodies.

46. On the activities of serum and liver cholinesterase in circumscribed liver damage. By TAKEO KITANI, TERUNORI KASHIMURA AND TADAMITSU ABE (*From the 2nd Clinic of Internal Medicine, Osako University, Osaka*). For the purpose of confirming the relationship between cholinesterase and liver, the activities of serum and liver cholinestrase were examined manometrically with partially hepatectomized rats and the rabbits containing both true and pseudo cholinesterase in serum (as designated by Mendel). The activity of the serum enzyme of partially hepatectomized rabbits decreased on the 2nd day, which could be restored on the 12nd day. The most marked decrease in the activity of the serum enzyme was observed for 0.01 M benzolycholine (Bch), slightly for 0.025–0.05 M acetylcholine (Ach), and far less for 0.0025–0.005 M Ach and 0.02 M acetyl-β-methylcholine (Mch). The serum enzyme varied in its activity by partial hepatectomy and is considered to be specific to Bch and profoundly related to liver damage. The activity of cholineste rasd of the survived rabbit liver did not obviously change for 0.025 M Ach, Bch, and Mch. In the survived rat liver, the activity of enzyme for 0.025 M Ach was behalved in 1–2 days after the operation in conspicuous cases, but it was restored in 3–7 days postoperatively.

47. Chemical studies on Fugu poison. By JUNJI NAGAI (*From the Department of Medical Chemistry, Kyshu University, Fukuoka*). The poison contained in the ovaries of Fugu (Globefish, *Spheroides rubripes*) was isolated. A preparation had the toxic activity at its minimum lethal dose of 0.02 γ/g. of mouse Body weight. In neutral solution the preparation had an absorption band at 265 mμ, which disappeared in alkaline medium, but again appeared on neutralization. Some flocculent precipitates were settled by heating in alkaline solution in a few minutes. With the heat-treated preparation, the band at 265 mμ could not be reversed by neutralization.

48. Studies on the anterior pituitary hormones. II. Isolation of gonadotropic hormones. By KENICHI TAKEDA, HIDEO OOTSUKA AND YUKINAO NODA (*From the Shionogi Pharmaceutical Institute, Osaka*).

49. Demonstration of an apparatus for the determination of dissolved gas. By KEN IWASAKI AND TSUTOMO MINOYA (*From the Department of Medical Cehmistry, Kanazawa University Medical School, Kanazawa*).

50. A photometric method for the determination of sugar in tissues and serum. By SADAO KOBAYASHI (*From the 2nd Department of Medicine, Kobe Medical College, Kobe*).

51. A new method for the determination of calcium and magnesium in serum. By FUMIMASA YANAGISAWA. (*From the Laboratory for Medical Sciences, Tokyo*). A new method has been devised for the determination of Mg and dialyzable Mg in serum, using the author's previous method for Ca dtermination. (a) An 0.1 ml. portion of serum is diluted to 0.5 ml. and to the diluted serum (1 : 4), 2 ml., of $2.5\times10^{-4}M$ sodium chlorophenolazodihydroxynaphthalene disulfate and 2.5 ml. of 0.2 *N* NaOH are added. After standing for 10 minutes, the extinction, E, is determined with an electrophotometer (Leitz) by using a filter (640 mμ). The value thus obtained represents the amounts of Ca plus Mg. (b) For the determination of Ca alone, 0.5 ml. of the diluted serum (1 : 4)is mixed with 2 ml. of the dye solution, 2.5 ml. of 0.2 *N* NaOH and 0.25 ml. of 10*N* NaOH, successively; and E is determined photometrically. (In such a strong alkaline solution Mg develops no color with the dye). (c) For the determination of dyalyzable Mg, 0.1 ml. of serum is well mixed with 0.4 ml. of 0.2*N* NaOH, and let stand for 5 minutes; to the mixture are added 2.0 ml. of the dye solution, 2.1 ml. of 0.2 *N* NaOH, and 0.4 ml. of distilled water; and the value of E is estimated photometrically. Thus obtained value represents the sum of Ca and undialyzable Mg. From the values obtained in procedures (a), (b) and (c), the amounts of Ca, total Mg, dialyzable and undialyzable Mg can be calculated.

Torao Kawaachi: Is there any influence of protein on the color development in you method of Ca-determination? I should like to ask, why a filter-640 mμ is employed.

Fumimase Yanagisawa: There is no influence of protein. From the absorption spectra of the developed color of Ca with the reagents, it is the most advantageous to use a filter-620 mμ. In our laboratory, a filter-640 mμ of Leitz electrphotometer is availabe for convenience at present.

Takeshi Yamada: In what ratio Ca ions are bound with the dye? How much dye should be added to serum?

Fumimase Yanagisawa: The ratio of the dye to Ca is 2 : 1. For 0.1 ml. of serum, it is most preferable to add 2 ml. of the $2.5\times10^{-4}M$ dye.

52 A new titrimetric micromethod for the determination of calcium and magnesium in blood serum. By TORU ISHII (*From the Clinical Laboratory of the First National Hospital of Tokyo Tokyo*). The present method for the determination of serum Ca and Mg is a modification of the method of Schwarzenbach *et al.* proposed for the determination of water hardness in 1947. The principle of this method is as follows; at pH 10–11, Ca and Mg ions are bound with disodium salt of ethylenediamine tetraacetic acid quatitatively and sensitively. The dye, Eriochrome Black T, is used for an indicator; this dye changes clearly from a wine-red in the presence of free Ca and Mg ions to a greenish-blue in the absence. *Reagents*: (1) *N*/20 Disodium ethylenediamine tetraacetic acid (ETA), stock solution. Dissolve 9.31 g. of ETA in 1000 ml. of water. For use, take 2 ml. of stock solution in an 100 ml.-volumetric flask and dilute to the mark with water. It should be prepared freshly once a week. (2) Indicator solution. Dissolve 0.5 g. of Eriochrome Black T (EBT) and 4.5 g. of hydroxylamine hydrocholoride in 200 ml. of methanol. (3) Buffer solution. Dissolve 67.5 g. of ammonium chloride in 570 ml. of 28% liquid ammonia and add water up to 1000 ml. (4) Buffer-indicator mixture. To 1 ml. of buffer solution add 3 drops of indicator solution and mix. It should be prepared immediately before use It is stable for 2 hours. The color of this mixture should be deep blue. (5) Saturrated ammonium oxalate solution. Approximately 4 g. per 100 ml. solution. (6) Standard Ca solution. Dissolve 24.99 mg. of pure $CaCO_3$ in a small quantity of 6*N* HCl in an 100 ml.-volumetric flask and add an aliquot of sodium acetate solution to pH 4–6 and then dilute with water to the 100 ml.-mark. This is a 10 mg. per cent Ca solution. *Procedure*: Each 0.1 ml. of serum is transferred to 2 Kahn's test tubes. Into the first tuqe a drop of saturated ammonium oxalate solution is added and then stand for 30 minutes longer. A 0.4 ml.-portion of the buffer-indicator mixture is added to the second tube (serum 0.1 ml.) and then tit. rate with ETA solution from a microburette to bluish-green endpoint (ETA, a ml.). Then, the third tube containing 0.1 ml. of the standard Ca solution and the first tube are titrated by the same procedure (There are b ml. and c ml., respectively). *Calculation*: It is calculated with a ratio of titration values. (1) (a ml./b ml.)×10=Ca plus Mg, mg./100 ml. (It calibrates as Ca); (2) [(a ml.-c ml.)/b ml.)]×10=true Ca, mg./100 ml; (3) ((1)—(2))×0.607=Mg, mg./100 ml.

53. Colorimetric microdetermination of blood magnesium and sodium. By TAKESHI YAMADA (*From the Department of Biochemistry, School of Medicine, Chiba University, Chiba*). The new methods for the determination of blood Mg and Na have been devised as follows: (a) An 0.1 ml.-portion of serum is deproteinized (Folin-Wu's method). From the filtrate Mg is precipitated as salt of Tropaolin 00. This salt precipitate is washed with acetone-ether for removal of Ca salt, dissolved in 1 ml. of conc. H_2SO_4, and diluted to 10 ml. After standing for 9 minutes the extinction coefficient, E, is determined with Pulfrich's photometer in a 10 mm.-cuvette by using a filter S_{53}. ($E = 1.002/0.5 \times 10^{-7}$ *M* Mg). (b) Na is directly precipitated as pyroantimonate from 0.1 ml. of serum. The precipitate is washed with 50% alcohol. The washed precipitate dissolved in 5 ml. of conc. HCl., and diluted to 10ml. A 2 ml.-portion of this mixture is pipetted into a test tube, to which 1 ml. of KI solution and 5 ml. of benzene are added. after vigorous shakings, the developed red color is measured by a colorimeter or by a photometer in a 10 mm.-cuvette using a filter S_{50}. ($E = 0.802/0.2 \times 10^{-4} M$ Na).

54. A micromethod for the colorimetric determination of acetone-bodies. By YASUSHI KOIDE, TOMI MORITA and HIROSHIGE MUKAIYAMA (*From the Department of Biochemistry, Niigata University School of Medicine, Niigata*). A modification of the Greenberg and Lester's method for the determination of acetone·bodies in blood and urine is proposed. The necessary amount of the sampleis 1 ml. for each determination. The present modification extends its usefulness to each fraction of acetone-bodies and makes the analysis more sensitive and simple. The determination is carried out as follows: (a) *Acetone*: 1 ml.of the sample is mixed with 5 ml. of distilled water, and to the mixture 10 ml. of 2.5% $ZnSO_4$ and 10 ml. of 0.15% $Ba(OH)_2$ are added. 20 ml. of the supernatant fluid, is mixed with 2 ml. of 0.5% 2, 4-dinitrophenylhydrazine hydrochloride and 2.5 ml. of CCl_4. The mixture is shaken with a shaking machine vigorously. The CCl_4 layer which contains acetone-hydrazone is mixed with 6 ml. of ethanol and 0.1 ml. of 2 *N* NaOH. The resulting pink color is read by Pulfrich stupfenphotometer in a 2 cm.-cuvettes light path, using a S_{43}- or S_{47}-filter. (b) *Acetone and Acetoacetic Acid*: 20 ml. of the supernatant fluid are mixed with 2 ml. of 2 *N* H_2SO_2. Then the tube is sealed off at the neck and heated for 3 minutes in a boiling water-bath. By this procedure acetoacetic acid is decomposed to acetone. After cooling, 20 ml. of the content in the tube is pipetted out, and the acetone is estimated. (c) *Acetone, Acetacetic Acid and β-Hydroxybutyric Acid*: At the first step of the estimation, 20 ml. of the supernatant fluid is treated as in Exp. (b) to decompose the acetoacetic acid to acetone. At the second step, 20 ml. of the solution in the above sealed tube is mixed with 2 ml. of $K_2Cr_2O_7$-H_2SO_4 solution, transferred to another sealed tube, and heated for 30 minutes in a boiling water-bath. 91% of β-hydroxybutyric acid is decomposed to acetone by this procedure. To 20 ml. of this solution cooled in ice-water, 1 ml. of 7.5% Na_2SO_3 and 7 ml. of 7.5 *N* NaOH are added. The acetone is then estimated as in Exp. (a).

55. Schiff's reaction of human urine. By YOSHIYASU TAKAHASHI (*From the Biochemical Laboratory, Toho Medical College, Tokyo*). In order to analyze Schiff reaction-positive substances in human urine, this reaction is applied to various related substances. Piror to the addition of the Schiff's reagent, urine is adjusted to pH 5.0-5.4, kept at 25°, and mixed with alcohol (final concentration, 20%). After standing for 50 minutes, the color intensit is ready by a colorimeter. The Schiff reaction of urine decreases in its intensity, which turns to negative in cases of starvation and of an excess vitamin B_2 administration; but it rather increases in its intensity after the administration of sugar in parallel with the contents of pyruvic acid in urine and blood; a remarkable increase in the color intensity is observed after vigorous exercises. Among many related substances, the reaction *in vitro* is positive with pyruvic acid, creatinine, glucuronic acid, and acetaldehyde; weakly positive with uric acid; and is negative with acetone, acetic acid, urea, lactic acid, glucose, fructose and phenol.

56. A method for the microdetermination of glutamic and aspartic acids. By SHOZO TANAKA AND FUMIKO KATSUKI (*From the Biochemical Laboratory, Facultyof Science, Kyoto University, Kyoto*). Glutamic and aspartic acids were separated from each other by use of anion exchange resin (Amberlite IR-4B). They were then separated by paper partition chromatography using a mixture of pdenol—acetic acid—water (65 : 10 : 25) as a developing solvent. The spots developing with ninhydrin reaction were determined photometrically. Determination range: 5-30 γ amino acid; recovery, 90-94%. Applying this method to the determination glutamic and aspartic acids in the leaves of rice plant at their several stages of the growth, almost equal amounts of these two amino acids were found to be always contained in the leaves. The content of total dicarboxylic amino acids was, however, found to become maximum at the end stage of the growth.

57. The determination of guanidine compounds in some organisms by paper chromatography. By SHIN'ICHI SHIBUYA, SATORU MIKAMI, GORO TSUCHIHASHI AND TETSURO

TAGAWA (*From the Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka*). For the determination of known guanidine compounds and the detection of the new ones, paper chromatographic analysis has been applied to some plant and animal muscle extracts on strips of Toyo filter paper, No. 5B, by the one-dimensional descending method, using a mixture of *n*-butanol, acetic acid, pyridine and water (4 : 1 : 1 : 2) or that of *n*-butanol and 6 *N* HCl (7 : 3) as the developing solvent. The guanidine compounds contained were identified by Sakaguchi's reaction, Na nitroprusside—K ferricyanide reaction, and Jaffe's reaction. Rf-values were determined by the spot dilution method. The foloiwing results were obtained: In most pulses only arginine was found. The distribution of canavanin was narrow and almost limited to jack bean. In addition to canavanine the jack bean contains a new guanidine compound which showed positive Sakaguchi's reaction, and picreate of which decomposed at 244–5°. In muscles of the vertebrates, especially in fishes, creatine was found; arginine was present in the globe fish and in the sea-eel. In muscles of the invertebrate no new guanidine compound was so far found. In shells, however, in addition to arginine, there was founda guanidine compound, having the same Rf-value as octopine, but it has not yet been exactly confrmed.

58. Studies on the chlor ion activity of body fluid. By TOSHIO SAKAI (*From the 1st Department of Medicine, Osaka University, Osaka*). The determination of Cl ion by the method previously reported is unsatisfactory for the purpose of the determination of free-Cl-ion in body fluid. The author has devised an apparatus equipped with a AgCl-electrode by means of which the determination of Cl ion can be readily made poteniometrically. With this apparatus, the variation of the potential difference of saline solution was determined under various conditions. When various ions, such as those of protein, carbohydrate and lipid were present in the solution, the Cl-ion concentration was found to decrease, and under these situations the amounts of bound- and free-Cl ions could be separately determined. This method was aplied to the determination of the Cl-ion activity in the serum and urine of normal rabbits and human individuals. After the administration of saline solution, the change in the Cl-ion activity in serum, urine and subcutaneous tissue fluid were determined for a clue of the mechanism of Cl metabolism.

59. An application of the ion exchange resins for removal of salt ions contained in serum protein fractions. By YASUO MATSUMURA (*From the Labotatory of Clinical Chemistry, Tokyo Medico-Dental College, Tokyo*). The mixed ion exchange resin of IRA-410 and IR-120 (2 : 1) have been applied to remove salt ions from protein fractions as follows: Albumin solution prepared by salting out method is dialyzed for 24 hours. The dialyzed albumiin solution is passed through a column filled with the mixed resins. In case of globulin the solution is shaken in a glass flasket with the mixed resins. By this method, the loss of albumin and globulin are 4 and 5%, respectively. The proteins are not practically adsorbed on the resins. That the horse serum proteins are not denatured by the resins has been proved by examining the resin-treated albumin or globulin with the following tests: weak alkaline biuret reaction (Araya), formol gelification (Miyamoto-Suzuki) electrophoresis, viscosity, and recrystallizability.

60. Enzymatic formation of uric acid from the deproteinized solution of human red cells. By TORAO KAWACHI (*From the Mitsui Medical Research Institute for Industry, Inatsuki-machi, Fukuoka*). The deproteinized solution of human red cells shows the characteristic absorption bands of purine derivatives at 257 and 231 mμ. The enzymaticmetabolism of these purine derivatives were studied spectrophotometrically. A considerable amount of uric acid was found in rat and guinea-pig sera. After incubating whole blood or/and plasma of rat the uric acid content increased greatly, which was most possibly derived from itsprecursor (purine derivatives) by the action of rat plasma xanthine oxidase. When rat serum was incubated with any one of the substances (adenine, adenosine, guanine, guanosine, RNA, DNA, and hypoxanthine) for 1–6 hours, the typical absorption bands of uric acid were readily detectable, with all mixtures, except the adenine-plasma mixture. These results indicate that nuclease, nucleotidase, adenosine and guanosine deaminases, guanase, nucleosidase and xanthine oxidase are present in the rat plasma. With a mixture of rat plasma and deproteinized human red cell solution, the absorption bands of purine derivatives—chiefly of adenylicacid—were converted into those of uric acid. In view of the existence of the above-indicated enzymes in the mammalian tissues, the *in vivo* formation of uric acid or allantoin may be considered to be partly due to the enzymatic conversion of purine derivatives of the mamalian red cells.

61. The influence of catalase upon pancreatic amylase. By MUTSUO UMEDA (*From the 2nd Clinic of Internal Medicine, Osaka University, Osaka*). Catalase was found by the present author in the duodenal juice. It was confirmed that the catalase existed mostly in the pancreatic juice by the experiments with dogs or rabbits. The addition of crystalline beef liver catalase to the pancreatic juice increased 2–3.3 times the pancreatic amylase activity (determined by Willstatter and Schudels method) comparing with that of pancreas juice alone. This activation of amylase by catalase was entirely suppressed by heating the reaction mixture.

62 On the internal secretion of salivary glands in view of the variation of blood-amylase. By YASUSHI NARABAYASHI (*From the Department of Biochemistry, Tokyo Medical College, Tokyo*). It was showen in 1928 by Ogata *et al.* that the internal secretion of salivary glands took place through the re-absorption of saliva at the root of the salivary gland ducts (so-called part of stripe-cell). Recently, in addition to the above theory, the author published a new hypothesis. If the theory is correct, ptyalin in saliva must be transferred into blood, thus, exerting several influences upon the variation in blood-amylase. Keeping this hypothesis in mind, the present study has been carried out, using healthy rabbits, with the purpose of confirming the function of internal secretion of the salivary glands through the observation of blood-amylase variation, which would appear as the results of several kinds of operations on the salivary glands and excretory ducts of the glands. It is indicated by the experimental results that the retention of saliva due to the tying up of the excretory ducts of parotid glands, or the compensative hyperfunction of the parotid glands due to the extraction of the submaxillary glands, caused the increase in blood amylase These facts show, at least, the possibility of transferrence of ptyalin to the blood through the root of the salivary gland ducts.

63. Studies on the mechanism of quinoline-dehydrogenase. By RYOJI ITOH and YUKICHI HASHIMOTO (*From the Department of Medical Chemistry, Nihon University, Tokyo*). In order to clarity the mechanism of the quinoline-dehydrogenase, thefollowing experiments were carried out. By the action of purified enzyme, quinoline and quinoline iodomethylate were oxidized to carbostyril and N-methyl-alquinolone, respectively. For the oxidation of 1 mole quinoline, 0.491 moles oxygen were consumed: where 2 active hydrogen atoms were found to be left from 1 molecule of quinoine. If the various substrates underwent the oxidation, the iodomethylation was easily carried out; while the substituted derivertives at the 8th position of the quinoline nucles notably hindered the action of the enzyme. The action of the enzyme was shown to be different with varying buffer substances; especially in case of the oxidation of substrates without the substituted radical at the N atom of quinoline nucleus, such as quinine, hydrocinchonidine, quinoline *etc.*, the action of the enzyme was more notably retarded in borate buffer than in phosphate one. But this was not the case with the substrates, N atom of which was iodomethylated, as quinolineiodomethylate. The similar results were obtained whith xanthine dehydrogenase; but in case of the oxidation of aldehydes, (acetaldehyde, crotonaldehyde, benzaldehyde, anise aldehyde, cinnamaldehyde, *etc.*), by aid of the quinoline-dehydrogenase, such difference were not observed.

64. The contents of SH groups and succinic oxidase activity of isolated rat-liver mitochondria. By TETSUO HAYASHI, ZYUNKICHIRO HIRADE, MIEKO YAMADA, and HIDEKO KIMURA (*From the Department of Biochemistry, the National Institute of Public Health, Tokyo*). Some fundamental experiments has been carried out to study the correlation between the decrease in SH- groups of the isolated rat liver mitochondra kept in contact with some selected SH reagent and chiefly the succinic oxidase or *a*-ketoglutaric oxidase activity. The SH content is measured by Barron's ferricyanide method or the amperometric titration. *p*-Chloromercuric benzoate, $HgCl_2$ and $CuSO_4$ decrease both the SH contents and the enzyme activity at the same rate. The oxidizing reagents, such as tetrathionate and chloropicrin, give the similar results. The partially inactivated enzyme activity of mitochondria by the action SH reagents (the oxidizing, mercaptide-forming, and alkylating reagents) can be restored considerably by the addition of an excess amount of GSH, and the reduced SH contents in mitochondria are restored correspondingly. The results concerning the inhibition of the enzyme activity by these reagents indicate the reversible and specific blocking of the sluggish SH groups.

Junkichiro Hirade: I should like to speak of my opinion in regard with the present report. The additional studies will be carried out innear future on the "oxidative inhibition by X-rays". Such a probled will be related closely to the role of -SH in carcinogenesis. In this connection I might point out the interesting observation that the chromosome abbreviation due to X-ray is remerkabley reduced in N$_2$-atomosphers, and this fact might, according to my opinion, be accounted for on the basis of oxidation or non-oxidation of the -SH groups.

65. Studies on acylase. By TSUTOMU HOSODA (*From the Department of Medical Chemistry, Kyoto University, Kyoto*). By the action of the homogenates of fresh pig kidney with water (: 15), diglycine (GG) and acetylglycine (AG) were hydrolyzed completely within 4 hours' incubation. The acidity increments with GG determined by formol titration were twice as much as those with AG in a shorter time of incubation. The grade of hydrolysis of other peptides by this preparation was greater in the order of benzoylglycine (BG), benzoyldiglycine (BGG); but acetyldiglycine (AGG) was hardly hydrolyzed. In hydrolysis of BGG. AGG, and BG, the acidity began to increase only after 24 hours. To the kidney homogenates (1 : 1) acetone was added at the ratio of 30, 40, 45, and 50% (v/v) and, after centrifuging the formed precipitates, the activity of each corresponding supernatant was examined as follows: with the enzyme preparationby 50 % (v/v) acetone precipitation, the grade of hydrolysis of AG was 4 times as much as that of GG. These results indicate that this 50% (v/v) acetone fraction of enzyme shows the inverse activity of the original solution. The acetone dried powder from the supernatant fluid of the acetone fraction between 45 and 60% (v/v) had a very strong acylase activity in contamination with weak dipeptidare and minmum hippuricase activites.

66. On the mechanims of vitamin B$_1$ decomposition by aneurinase. III. The experiment to clarify whether or not pyrimidine part exists in the decomposition products. By KIKU MURATA and HIDEO IKEHATA (*From the Osaka Municipal University, Osaka*). In the reaction mixture containing vitamin B$_1$ and aneurinase of *B. thiaminolyticus* (M.M.), a free nucleus of NH$_2$-Pm (2-methyl-4-amino-5-hydroxymethyl-pyrimidine) and that of OH-Pm (2-methyl-4-hydroxy-5-hydroxymethyl-pyrimidine) molecules failed to be developed on the paper-partition-chromatogram using Dragendorff's reagent. But the presence of free thiazole was comfirmed. In order to ascertain whether or not OH-Pm and NH$_1$-Pm exist in the reaction mixture of B$_1$ and aneurinase, the above-mentioned paper partition chromatogram was examined by the spectrophotometric method. With the reaction mixture both OH-Pm and NH$_2$-Pm were not detected at the point of their Rf values, 0.4–0.5 and 0.5–0.6, respectively. It is thus, assumed that Pm spot is not developed on the paper, because of its combination with protein of aneurinase; otherwise its absorption on the same spot either with vitamin B$_1$ or with thiazole part.

Kiku Murata: It has been confirmed that the substance produced from vitamin B$_1$ by the attack of "Aneurinase I" does not undergo the hydrolysis, but it is rather presumed as Pm-X. It is of much interest to know that the substances isolated recently in pure state by Hennessy as we have been trying to elucidate the structure of Pm-X.

67. Studies on the thiamine-decomposing thermostable factor of plant. By YOSHITSUGU NOSE. TAKESHIRO OKAMOTO and EIICHI HASEGAWA (*From the Biochemical Institute, Kyoto Prefectural Medical College, Kyoto*). " Anti-thiamine factor " of fern is composed of two factors: a thermolabile factor (thiaminase) and a thermostable non-enzymatic factor (SF). Thiaminase is inactivated by heating at 100° for 30 minutes; while SF is inactivated only at below pH 6. These differences in their properties can be applied to the isolation of both factors separately from many edible plants. Thiaminase is found in many fern, horsetail and cockscomb. The enzyme is remarkably activated by aniline, but the thiaminase preparations of certain ferns (*Gleichvia glauca*, *Dycopodium clavatum etc*.,) are not activated. SF is detectable in almost all plants, and SF activity is generally stronger in leaves than in stems. SF is particularly strong in the leaves of *Pteridium aquilinum* and sweet potato. The activity of SF rises with the temperature. SF is dialyzable through cellophane membrane after heating, while the non-treated SF extract is non-dialyzable. Activating effect of aniline of SF is not attained. The activity of SF is also promoted by oxygen. By the action of SF, thiamine is oxidized to thiochrome, which is easily demonstrable by paper chromatography. SF preparations from two different sources are presumably identical with each other. SF occurs generally combined with protein in nature. Since SF is scarcely active at body temperature, it is probably insignificant for human nutrition except in case of thiamine-inactivation by cooking process.

68. Studies on the inhibitor of the natural decomposition of ascorbic acid contained in brain tissue. By TAKEHIKO SAKURAI (*From the Department of Biochemistry, Shinshu University Medical School, Matsumoto*). The addition of the aqueous extract of cow brain tissues showed the prevention of the autoxidation of ascorbic acid. In order to know the chemical nature of this active substance, the brain was first extracted thrice with acetone, and then thoroughly with ether. Both acetone- and ether-extracts were separately evaporated to dryness, and the respective residue was dissolved in alcohol. The emulsions separately obtained by the addition of water to each alcohol solution did not inhibit the autoxidation. The water extract of the non-extracted residue inhibited the autoxidation as strong as the original extract, but the suspension of the insoluble part of the residue had no such an effect. The water extract gave both color and precipitation reactions for protein, and its oxidative activity was thermostable (80° for 30 minutes). When the water extract was separated by dialysis into dialyzable and non-dialyzable parts, each fraction alone was inactive by itself, but on mixing them the activity enhanced in the original proportion. The non-dilyable fraction was thermolabile, gave protein reactions, and decomposed by heating at 80° for 20 minutes; trysin inactivated this fraction. On the other hand, the dialyzable fraction was thermostable (100°, 30 minutes). The non-dialyzable fraction was insoluble in water, but an active transparent solution was obtained by dissolving both fractions simultaneously. The solubility of the non-dialyzable fraction was increased by the addition of NaCl or KCl, and at the same time the anti-autoxidation activity was recovered. From these results it may be concluded that the inhibitory substance of cow brain tissue is a substance of protein nature, consisting of the non-dialyzable protein and of the dialyzable inorganic compounds. The latter is required for the solubility of the protein moiety as well as for the anti-autoxidation activity.

69. Variation of vitamin B_2 content in the rabbit liver and kidney with the development of the animal, and the seasonal effect on its content. By DAIZO YOSHIDA (*From the Department of Biochemistry, Iwate Medical College, Morioka, Iwate*). In the developmental stage of rabbits (from embryonic stage to maturity), the quantitative determination of the vitamin B_2 content of liver and kidney together with three forms of vitamin B_2 (*i.e.*, free flavin, FAD, and FMN) were carried out by lumiflavin method and paper chromatography. The vitamin B_2 content in liver and kidney was almost invariable in the latter half of embryonic period, but it rapidly increased after birth this increment being perhaps due to the dffect of milk diet; thereafter, vitamin B_2 content increased with the growth, thus reaching the maximum at the stage of vigorous development At each stage of the growth the ratio of the vitamin B_2 content to the body weight was nearly constant. During about 60 days after birth, the vitamin B_2 content in liver and kidney per body weight increased regularly, and reached the maximum. These results will indicate the increasing demand of vitamin B_2 at the sages from infancy to maturity. The distribution of three forms of vitamin B_2 in percentage did not change remarkably in all stages of the growth. Total vitamin B_2 and its three forms showed an inevitable consequence with season.

70. On the fluctuation of vitamins B_1 and B_2 contents in giant salman der and sea-turtle eggs during their development. By MASAJI TOMITA, KOYATA HAMADA, YOSHIKO MANABE AND MASAMI SASAKI (*From the Biochemical Institute of Kobe University and the Research Laboratory of Biofermin Co., Inc., Kobe*). This paper has been published in *J. Biochem.* **39**, 299 (1952).

71. On the crystallographic differences between riboflavin and araboflavin. By KAZUO HOTTA, HACHIRO SAKATE AND TADASHI MORISHIMA (*From the Department of Biochemistry, Nagoya University School of Medicine, Nagoya*). The crystallographic differences between riboflavin and araboflavin have been studied. The two flavins have different pentyl alcohol groups. In X-ray diffraction patterns, the Debye-Scherer patterns are obtained using Cu Ka radiation filtered by a Ni foil for about 7 hours of the exposure time. There are 8 rings in the photographs of two flavins, in which the spacings and intensities are different. By using a polarization microscope, both flavins were found to belong to orthorhombic system; they showed different 3 principal refraction indices, resulting in the angle differences of about 2° between 3 angles of optical axes.

72 Isolation of a new cysteine desulfhydrase from rat liver and the mechanism of the enzymatic reaction. By MASAMI SUDA AND EIZO KI-

ZU (*From the Department of Bacteriochemistry, Research Institute for Microbial Diseases, Osaka University, Osaka*). The present study is concerned with the separation and the partial purification of the cysteine desulfhydrase from rat liver. The method of isolation of the enzyme is as follows: Acetone-dried powder of rat liver is extracted with 3 volumes of distilled water; the extract is centrifuged, and the enzyme in the supernatant fluid is then adsorbed on calcium phosphate gel, and finally eluted for 30 minutes with $M/5$ phosphate buffer at pH 8.0. To the eluate the ice-cold acetone is added (33 vol.% finally), centrifuged, and the supernatant is mixed with ice-cold acetone (60 vol.% finally). This procedure of acetone additions is repeated 2 more times, and the finally precipitated enzyme is washed with ether, and dried immediately. The dried preparation is dissolved in $M/15$ phosphate buffer at PH 7.4 and dialyzed for 20 hours against distilled water containing $10^{-3}\,M$ ascorbic acid. The dialyzed solution contains fairly purified cysteine desulfhydrase. The enzyme catalyzes anaerobically the formation of H_2S, NH_3 and pyruvic acid from cysteine in equimolecular ratio, exhibiting its full catalytic activity in the presence of both pyridoxal phosphate and inorganic phosphate. By measuring the amounts of NH_2, H_2S and pyruvic acid liberated in the enzyme reaction with cysteine, it has been found that NH_3 is first split off, and the curves of pyruvic acid and H_2S formations are similarly S-shaped. Arsenate inhibits the production of both H_2S and pyruvic acid completely by competing with the inorganic phosphate present, but does not affect the splitting off of NH_3. The enzyme preparation is inactive for L-serine and L-alanine, end contains no L-amino acid oxidase. From these facts it is reasonably considered that cysteine may be first deaminated and then desulfurated. In view of the above facts, *a*-amino acrylic acid can not be considered to be the true intermediate. Since semicarbazide and other carbonyl inhibitors simultaneously inhibit the formation of reaction products (NH_3, H_2S and pyruvic acid), pyridoxal phosphate might have a role in the desamination of cysteine.

73. The mechanism of the activation of *a*-chymotrypsinogen to *a*-chymotrypsin. By SHIRO AKABORI, NAOICHI SAKOTA AND KO OHONO (*From the Institute of Biochemistry, Faculty of Science, Osaka University, Osaka*) Crystalline *a*-chymotypsinogen prepared from fresh pancreas (Northrop and Kunitz method) was activated at 5° at pH 7.6 (0.5 M phosphate buffer) in the presence of trypsin; and after 48 hours, crystalline *a*-chymotrypsin was obtained together with three kinds of polypeptides which were detected by paper partition chromatography. The obtained polypeptides were found to be consisting of 3–6 amino acid residues. On the other hand, the hydrazinolysis of crystalline *a*-chymotrypsinogen and *a*-chymotrypsin demonstrated that at the carboxyl end both proteins had the same series of theree amino acids: glycine, alanine, and aspartic acid. From the results obtained it can be concluded that the hydrolysis of the peptide bonds near the amino end group is involved in the activation of *a*-chymotrypsinogen to *a*-chymotrypsin.

74. Studies on the hemolytic and agglutinating factors of the socalled-ricin fraction. By KIMIE KUSUNO-

KI (*From the Department of Biochemistry, Tokyo Medico-Dental University, Tokyo*). Both hemolytic and agglutinating factors was found to co-exist in the so-called ricin fraction (Moriyama). The author could separate them from each other by extraction at suitable pH. Hemolytic factor was extracted with alcohol-ether from the precipitate obtained from alkaline extracts of castor-bean by adjusting to pH 5.4 or by mixing with trichloracetic acid. In the latter case the hemolysing power was higher in free state than in the presence of protein; but this lipid hemolytic factor is unlikely to be a phospholipin or a neutral fat, because of its solubility in acetone and insolubility in alkaline ether. Hemolysis by this hemolytic factor occurred only when the concentration ot the factor was high in the initial stage of the reaction, but, as time passe by, hemolytic factor could induce perfect hemolysis even at its lower concentrations. It is noteworthy, however, that hemolysis did not occur at all when the concentration of hemolytic factor was lower than a certain definite value even after a long standing. The same tendency has been observed with oleic acid. Moreover, hemolytic actions of both oleic acid and lipid of ricin were inhibited by cholesteriol. From the seresults it is concluded that the hemolytic factor may posibly be a fatty acid. Agglutinating factor obtained by acid extraction of defatted castor-bean could be partially precipitated in alkaline solution. In this case agglutinating power per N of the supernatant was higher than that of the precipitate. Precipitates were obtained by adding an equal volume of acetone to the supernatant at varying pH between 3 and 8. Aggutinating power per N of the precipitate obtained at pH 4 was the greatest.

75. The chemistry of the lipid of posthemolytic residue or stroma of erythrocytes. III. Globoside, the sugar-containing lipid of human blood stroma. BY TAMIO YAMAKAWA AND SHIZUE SUZUKI (*From the department of Chemistry, the Institute for Infectious Diseases, the University of Tokyo*). As metioned in the 1st Report (*T. Biochem.*, **38**, 199 (1951), palmityl sphingomyelin and apparently a new glycolipid, named "hematoside," have been isolated from the ether-insoluble lipid fraction of equine blood stroma. The hematoside gives purple color with the Gial's orcinol test; and it is found to be composed of lignoceric acid, sphingosine, galactose and prehemataminic acid in the ratio of 1 : 1 : 2 : 1. For the last component the purple color reaction is responsible. A glycolipid from the human blood powder obtained by Klenk method gives, however, no purple color with Bial's reagent, but it contains chondrosamine. Since the source material used by him was aged, deterioration might have occurred; in other words, hemataminic acid might have been converted to chondrosamine by enzymatic action. The authors prepared 250 g. of lyophilyzed human blood stroma and extracted with organic solvent. A glycolipid was obtained, which differed entirely from hematoside. For the sake of conveniences that the term "globoside" was proposed to designate this substance. The colorless globoside contains about 10 per cent of chondrosamine but no hemataminic acid; it has dextrorotatory power and microscopically show's perfectly round, homogenous globules. In view of the analytical data, the authors are under the impression that globoside is a complex or a mixture of lignoceryl (or nervonyl)-sphingosine-digalactoside and lignoceryl (or nervonyl)-sphingosine-acetyl-chondrosamine-digalactoside.

76. The chemistry of the lipid of posthemolytic residue or stroma of erythrocytes. IV. Distribution of lipid-hexosamine and lipid-hemataminic acid in the blood corpuscles of various species of animals. By TAMIO YAMAKAWA and SHIZUE SUZUKI (*From the Department of Chemistry, the Institute for Infectious diseases, the University of Tokyo, Tokyo*). Globoside, a glycolipid of human blood corpuscles, contains chondrosamine; whereas hematoside, a corresponding glycolipid of equine blood corpuscles contains hemataminic acid instead of chondrosamine. Thus it has been revealed that the blood corpuscles glycolipids of these two mammals are different from each other in basic component. The distribution of these two substances in the blood cells of various species of animals was tudied. An appropriate amount (10-80 mg.) of lyophilized stroma was extracted for 2 hours with $CHCl_3$-CH_3OH in a micro extracter, the extract was hydrolyzed with 2 *N* HCl for 14 hours, and the hexosamine content in this hydrolysate was estimated by Blix's method. Hemataminic acid was also determined by the method of Klenk and Langerbeins. (a) Hexosamine content: human, 200-360; goat, 300, sheep, 200-260; bovine, 170; chicken, 10; dog, 60; equine, 25 mg. per 100 g. dry weight of stroma; (b) Hemataminic acid content: human, goat, sheep, and chicken, 0; bovine, 350; dog, 440; equine, 430 mg. per 100 g. (c) Lipid-hexosamine is present but in less amount in white matter of brain than in gray matter; while hemataminic acid only in gray matter.

77. Micro-and ultramicromethods for lactic acid determination by means of azotometry. By NOBU HATTORI (*From Biochemical Laboratory, Faculty of Medicine, Kanazawa University*). Quantitative determination of lactic acid from 250 to 3.6 μg. can be performed by the azotometric procedure using a specific apparatus of oxidation-and-distillation. Iodoform reaction can be applied, in spite of Fürth and Charnass experimental data which indicated this reaction as very unsatisfactory one. Hagedorn procedure is proved useful in lactic acid estimation as well as in blood sugar. But in case of muscle, the only depronizing procedure is Scenck's method. Distillation finishes in 3 minutes and within 5 ml. of distillate. Water vapor distillation could be used.

78. Seasonal influence upon the ornithuric acid synthesis in avian body. By AKIRA WATANABE (*From the Biochemical Institute, Kobe Univesitry, Kobe*). Urine and feaces were collected without separation from fowl, and the amount of ornithuric acid was determined by Jaffe's method. It was found that the excretion of ornithuric acid was very low in summer, but it increased gradually from autumn to winter, and reached the maximum value in March.

79. Effect of sulfur compounds on bacterial vitamin B_1 synthesis. By HIDEO TERADA (*From the Department of Medicine, Niigata Universitry School of Medicine, Niigata*). To clearify if the volatile organic S compounds, such as diallylsulfide, diallyldisufide, isothiocyanic acid ester, have specific effect on bacterial vitamin B_1 synthesis or not, the effect of various S-compounds on vitamin B_1

production by bacteria was studied. *B. coli* and *Staphylococcus aureus* were cultivated in the normal broth for 24 hours at 37°, and the content of vitamin B_1 in these cultur solutions was determined. The organic S-compounds were found to be utilized effectively as the material for vitamin B_2 synthesis; especially thiazole was utilized by *Staph. aureus* with the best result. Inorganic S compounds were well utilized by *Staph. aureus*, but not by *B. coli*. It was hardly recognized that volatile substances of *Allium* plants and of *Crucifera* plants were superior to the other orgnic S-compounds. The increase in vitamin B_1 synthesis is not due to the fact that these S-compounds acelerate the bacterial development, but rather to the fact that S in them serves directly as the material for vitamin B_1 synthesis.

80. Studies on the second type of bacterial thiaminases. By YOSHITSUGU NOSE and KIYOSHI UEDA (*From the Biochemical Institute*, *Kyoto Prefectural Medicineal College*, *Kyoto*). The thiaminase preparation of *Bacillus aneurinolyticus* Kimura et Aoyama (BA) differs in many enzymatic properties from that of *Bac. thiaminolyticus* Matsukawa et Misawa (BT). The most remarkable fact is that BT enzyme is activated by various nitrogeneous bases, showing the base exhcnage reaction of thiamine, in common with the thiaminases of shell-fish, fish and plant; on the contrary, the BA enzyme is not activated by the bases, but rather inhibited. By paper chromatography, the reaction products by the BA enzyme were found to be pyrimidylmethyl alcohol (Pm. CH_2OH) and thiazole moiety. BT enzyme decomposes thiamine into a thiazole moiety and a pyrimidine compound as a result base exchange reaction, but Pm. CH_2OH was not produced. By the action of BT enzyme as well as BA enzyme on thiamine and aniline, pyrimdyl methyl aniline (Pm. CH_2. Anil.) was found to be produced by use of paper chromatography. BA enzyme, however, unlike BT enzyme, can combine aniline with Pm. CH_2OH. But BA enzyme can not combine various base except aniline with the pyrimidyl moiety of thiamine. Since aniline definitely inhibits the BA enzyme in the experiment of a short period, it may be concluded that the combination of aniline with the pyrimidyl moiety by the action of BA enzyme takes place not dircetly, but rather secondarily by the second enzyme contained in BA enzyme preparation by combining aniline with Pm. CH_2OH which has been produced by thiaminase II of BA. Pm. $CH_2.NH_2$ was also demonstrated to be utilized as a substrate for the second enzyme. (The Subcommittee on Thiaminase Research of the Vitamin B Research Committee agreed to name the enzyme like that BT "Thiaminase I," and that of BA "Thiaminase II" in 1951.)

Kiku Murata: We clarified as the new phenomenon that the aneurinase II produced from *B. aneurinolyticus* (K.A.). not only stimulates the reaction (A), but also promotes the reactions (B) in the following scheme:

$$\text{Pm+Aniline} \xrightarrow{\text{AnII}} \text{Pm-Aniline.......(A)}$$

$$\text{Pm-Aniline} \xrightarrow{\text{AnII}} \text{Pm+Aniline. ... (B)}$$

The later reaction (B) is presumed as the reverse reaction that of reaction (A).

81. Studies on cobalt metabolism of *Bacillus subtilis*. By SHOZO TANAKA, YASUO SAWADA and TAKURO YAMAMOTO (*From the Biochemical laboratory*, *Faculty of Science*, *Kyoto University*, *Kyoto.*) The chemical constituents of *Bacillus subtilis* grown on Waksman's media containing 2 p.p.m. radioactive Co^{60}, and of the remaining media were precisely studied during their growth. Among them total Co contents in the unit weight of mycelia was found to increase with the growth. However, Co^{60} in the 75% alcoholic extract reached the maximum after 48 hours from inoculation. Moreover the amount of vitamin B_{12} was estimated by means of colorimetric determination of cyanide in cyanocobalamine. It was found that cyanocobalamine was synthesized in the bacterial bodies at their growing stage and was excreted into the media when autolysis began to take place.

82 On the chemical composition of *Streptomyces griseus*. By YUKI ITO, MASAE SAKAI AND TATSUZO FUJII (*From the Department of Biochemistry, Gifu Prefectural Medical College, Gifu*). Chemical analyses of a strain of *Streptomyces griseus* have been carried out for the contents of lipid, carbohydrate and base constitutents. Methanol extracts of 9.1 kg. of the wet fungi for 6 months at room temperature were concentrated to syrup; the bacterial resude weighed 650 g. (a) Ether extract of this syrup gave 74.0 g. of acetone soluble fat and 0.7 g. of phosphatide. After saponification of 41.0 g. of the crude fat gave 21.5 g. of fatty acid mixture, 2.4 g. of unsaponificable matter and 3.1 g. of glycerol. The mixture of fatty acids was separated into solid and liquid fatty acids by lead salt-alcohol method; from the former myristic and palmitic acids were isolated; and in the latter the presence of oleic acid was suggested. No sterol was detected from the unsaponificable matter (b) Hot alcohol extract of the syrup gave 3.5 g. of purified white crystal (m.p., 245–268°), having the composition of C 50.46; H 8.38; N 12.39 and P 0% and showing positive ninhydrin reaction, but Molisch reaction and all other color tests for protein were negative. This crystal is presumably an amino acid or its related substance. (i) From water soluble fraction, about 1 g. of polysaccharide was obtained as powder which liberated an uncommon reducing sugar by hydrolysis with dilute acid; this sugar gave phenylosazone (m.p., 129–131°) and the composition of C 64.79; H 6.57; and N 20.86%; lysine was isolated as its picrate, but any other base was not detected; paper chromatography revealed the presence of glycine, alanine, threonine, valine, cystine, glutamic and aspartic acids, but no free reducing sugar. (ii) Chloroform extract of the bacterial residue gave only a slight amount of wax-like substance. From these results it can be concluded that this fungus contains quite little amount of phosphatide and wax (bacteria usually contain them in greater amounts); that neither sterol nor mannitol was detected as the constituents (molds contain a great amount of them); and that the white crystal obtained by hot alcohol extraction is presumably an amino acid, which may be a characteristic constituent of this fungus.

83. Studies on the esterase of *Mycobacteria*. By YUICHI YAMAMURA, KATSUHIKO OGURA AND SHIRO IMAZU (*From the Toneyama Research Institute of Toberculosis, Osaka City Medical University, Osaka*). The lipase and esterase activities of mycobacteria (*Myc. tuberculosis hominis, et bovis*; *Myc. avium* and other non-pathogenic *Mycobacteria*) were determined by titrimetry (Willstatter *et al.*) and manometry (Ammon). Each strain of mycobacteria has a weak fat-splitting activity, but the activity for tributyrin (esterase) being strong generally. The esterase (tributyrinase) was extracted with $N/4$ NH_2OH as a cell-free preparation from the acetone-dried or frozen cells. The suspension of the resting cells of *M. avium* and its cell-free extract contained a thermostable esterase (not inactivated by heating at 100° for 10 minutes). It might be a peculiar feature of *M. avium* that this thermostable esterase was demonstrable in this organism. No thermostable esterase has been found in the other mycobacteria. The thermostable esterase of *M. avium* (Takeo strain) was purified by adsorption on kieselguhr, precipitation with $(NH_4)_2SO_4$, and with acetone, and dialysis, successively; the final preparation was obtained as a white, water soluble powder having 10-fold activity as much as the original extract, and showing positive Millon, xanthoprotein, biuret, ninhydrin and Molisch tests; the enzyme was inhibited by H_2O_2, I_2, atoxyl and quinine, but not by cysteine, ascorbic acid and amino acids.

84. Studies on the tricarboxylic acid cycle in tubercle bacillus. I. By MASAMICHI KUSUNOSE, EMI KUSUNOSE AND YUICHI YAMAMURA (*From the Toneyama Research Institute of Tuberculosis, Osaka City Medical University, Osaka*). Recently the authors have extracted a number of enzymes related to TCA-cycle except citric acid synthesizing enxyme system from *Mycobacterium tuberculosis avium* in cell-free state as follows: (a) Scuccinic oxidase is extracted by grinding the cell-paste with quarz-sand; The enzyme is remarkably inhibited by malonate or azide like the enzyme of animal origin; (b) Fumarase is readily extracted by rupturing the cells with alternative freezing-thawing. Furmarase is the most stable one against aging among TCA-cycle enzyme. Under the authors' conditions, this preparation converts 80% of fumarate to L-malate at 37° in 5 minutes; (c) L-Malic oxidase is separated by freezing-thawing, and grinding the cells. The enzyme requires neither methylene blue nor DPN, and is quites table against dialysis. This enzyme preparation catalyzes the oxidative degradation of L-malate to pyruvate and CO_2. This preparation also decarboxylates oxaloacetic acid; (d) As the citric acid-synthesizing-system, acetone-dried powder of the cells is used, and the formation of citrate from C_4-dicarboxylic acid, such as oxaloacetate, either L-malate or fumarate, and either acetate or pyruvate has been demonstraied (6.1 0.7 and 4.8 micromoles citric acid were formed from fumarate plus acetate, acetate alone and fumarate alone respectively, at pH 7.0 in 100 minutes); (e) Citric oxidase is extracted from the acetone-dried powder of the cells. The enzyme requires methylene blue, Mn, and DPN for its catalytic action. Either dialysis or acetic acid precipitation participates the enzyme into apoprotein and cofactors. By this enzyme citrate is oxidized to α-ketoglutarate and CO_2; (f) a-Ketogluaric oxidase is contained in the above extracts for citric oxidase preparation. The enzyme catalyzes the oxidation of a-ketoglutarate to succinate and CO_2. Thus the authors have convincingly established the occurrence of the enzymes at all steps of TCA-cycle in *M. tuberculosis avium*. In consequence, it is considered that TCA-cycle operates in the terminal respiration of tubercle bacillus.

85. TCA-cycle of tubercle bacillus.

By TAIJI SASAKAWA and YAEKO FUJII (*From the Department of Biological Chemistry, St. Paul's University, Tokyo*). In the previous report (*Symp. on Enzyme Chem.*, **7**, 55 (1952)) it was presumed that tubercle bacillus could oxidize citrate through the TCA-cycle. Recently it has come to clear that the bacillush has TCA-cycle like the higher organisms. The cells of avian tubercle bacillus grown on the glycerol broth agar medium oxidized citrate only after a long induction period, but the dried cells after washing rapidly oxidized citrate, *cis*-aconitate and α-ketoglutarate without a lag phase. The permeability effect may be concerned to the observed lag phase. Then the cells grown on the Kirchner's no-serum-synthetic liquid medium containing 0.25% citrate were found to be able to oxdize citrate rapidly. α-Glycerophosphate gave no effect on these cells, though it activated the citrate oxidation by the cells grown on the glycerol broth medium. The cell-free extract prepared from the acetone-dried cells dehydrogeneated citrate and under aerobic condition broke down citrate to α-ketoglutarate in the presence of methylene blue, upon which Mn^{++} and Mg^{++} gave the activating effect. This extract also decarboxylated oxalosuccinic acid, which was effectively activated by Mn^{++} and Mg^{++}. From these results obtained, it can be concluded that tubercle bacillus breaks down citrate to α-ketoglutarate through TCA-cycle.

86. Oxidative assimilation of glycerol by *Mycobacterium tuberculosis avium*. By KAZUO OKUNUKI and TAKEKAZU HORIO (*From the Biological Institute, Faculty of Science, Osaka University, Osaka*). Glycerol has been known to be a favorable material for the growth as well as for a substrate of the respiration of tubercle bacillus. For the perfect oxidation of 1 mole of glycerol required 7 atoms of oxygen. With the suspension of the bacillus cells, only about 2 atoms of oxygen were consumed for glycerol as a substrate. By use of colorimetric method for the determination of glycerol, the added glycerol as a substrate was found to be scarcely remaining. In view of these facts, glycerol must be utilized for the oxidative assimilation. By the addition of 2, 4-dinitrophenol or NaN_3 as an inhibitor for the oxidative phosphorylation, more than 5 atoms of oxygen were consumed per 1 mole of glycerol disappeared, It can be assumed that a part of glycerol may be expended in the respiration and at least some other part may be oxidatively utilized for the cell-material synthesis. Such an attitude of the cell-suspension towards glycerol was little affected by various antibiotics specific to tubercle bacilli (streptomycin, aureomycin *etc.*) slightly lowered the velocity of the oxidative assimilation. Malonate gave no inhibition of the oxidation of glycerol or succinate by the cell suspension. It may be assumed that malonate is oxidized by this suspension, rsulting in no inhibition of dehydrogenation. With malonate as a substrate, CO_2 evolution proceeded far more rapidly than O_2 uptake. Acetate was well oxidized by this suspension. Thus malonate is possibly decarboxylated into acetate at first, which is further degradated oxidatively.

Yuichi Yamamura: Yamamura, Sasakawa and Imazu (*Tuberculosis* (*Kekkaku*), **24**, 435 (1949))have found the oxidation of malonate by the suspension of resting cells of *M. avium* (Takeo strain). The oxidative assimilation was demonstrated by us when the suspension of *M. avium* was oxidizing glucose, glycine, alanine, lactate, acetate or glutamate. It is not suitable to use the resting cells of *M. avium* for the quantitative study, because *M. avium* is a very variable strain.

Junkichiro Hirade: It is not natural that such a strong inhibitor against the TCA cycle as malonate is oxidized at so fast rate, though there is no denying a possiblity of its slight oxidation. How do you feel the report of Yamamura *et al.* who observed the inhibition of malonate towards the TCA cycle of tubercle bacilli?

Kazuo Okunuki: By use of the cell suspension malonate is definitely oxidized. We observed that the succinic acid oxidation by muscle was inhibited by the same malonate sample. It was reported recently that an enzyme which degraded malonate into acetate and CO_2 existed in the skeletal muscle of aquatic fishes, carp *etc.* (K. Yamada and U. Suzuki: *T. Agr. Chem. Japan*, **25**, 290(1951); 373 (1952)). When we consier that the extraced enzyme is sensitive to malonate and that the cell has such an enzyme as the carp which degrades malonate, we may understand our results are reasonable. We intend to investigate whether this strain has really such an enzyme or not.

87. Some comparative aspects of the metabolism of trichomonads. By SUZUOKI-ZIRO AND HARUTADA NINOMIYA (*From the Research Laboratory, Takeda Pharmaceut. Ind. Ltd., Osaka*) The O_2-uptake by the suspension of *Trichomonas foetus* (*Tf*) and *Trichomonas vaginalis* (*Tv*) was measured manometrically at vaying O_2tensions. The high O_2 tension exerted somewhat stimulating influence on the autorespiration of *Tf*. *But* O_2 showed the remarkable inhibitory effects on the O_2-uptake by *Tv* in the presence of glucose. It is concluded that this inhibition is caused by H_2O_2 accumulated during glucose oxidation. *Tf* suspension possesses catalase activity (Q_{Kat}=about 800), while *Tv* had no activity at all ($Q_{Kat}=0$). Namely, *Tv* lost its catalase activity due to the long-continued parasitic existence *Tv* is not only more anaerobiotic, but also nutritioanally more exacting than *Tf*. These facts suggest that *Tf* may be wilder than *Tv* in the course of the evolutional adaptation from "free-living" to "parasitic."

88. Folic acid like and antagonistic activities of pteridine derivatives on *Streptococcue faecalis* and *Lactobacillus casei*. By HARUTADA NINOMIYA, EINOSUKE OHMURA, KATURA MORITA, JUNZO KATSURAGI AND NATSUKO TOKI (*From the Research Laboratory, Takeda Pharmaceutical Ind. Ltd., Osaka*). The following derivatives of pteridine, namely, 2,4,7-trihydroxypteridine-6-(carboxylic acid, isopropionic acid, and sec. isobutyric acid) (I), (II), (III), 2-methyl-4, 6, 7-trihydroxy-pteridine (IV), 1-methyl-4, 6, 7-trihydroxy-pteridine (V), 2-amino, 4, 6-dihydroxy-pteridine-6-(propionic acid, and acetic acid) (VI), (VII), 2-amino-4, 6-dihydroxy-7-ethyl-pteridine (VIII), 2-amino-4-hydroxy-pteridine-(6-carboxylic acid, and 7-carboxylic acid (XI), (X), 2-amino-4-hydroxy-pteridine-6-methylamino benzoic acid diethylaminoethanol ester (XI), were studied nutritionally with regard to their folic acid-like or antagonistic acivities on *S. faecalis* and *L. Casei*. On *S. faecalis* V, IX, X, and XI revealed folic acid-like actions, while I and IV were antagonistic against PGA. On *L. casei* XI showed weak folic acid-like activities, and the others scarcely any appreciable effect.

89, Bacteria creatine-splitting enzyme of bacteria, By TSUTOMU TAKEUCHI and ICHIRO KATSUMATA (*From the Department of medical Chemistry, Chiba University School of Medicine, Chiba*)

90, Biochemical studies on the metabolism of pentoses. I. On the utilization of several carbon sources by *Torula utilis*, By TATSUO SUZUKI and IWAO HAMATA (*From the Biochemical Institute, Mie Prefectural Medical College, Tsu*), The fact that *Torula utilis* can utilize pentoses as carbon sources has been already well recognized by many investigators, The following experiments were carried out to compare the ability of *Torula utilis* to utilize carbon sources by determining the proliferation of the cells, Culture medium was containing 0.7% $NH_4H_2PO_2$, 0.1% K_2HPO_2, 0.3% $MgSO_4$, 2% carbon source, and adjusted to pH 5.3. One loopful of the fresh yeast which had been cultured in the glucose medium was inoculated in each 5 ml. of the culture mediums, and incubated aerobically (50 ml. air/minute) for 18 hours at 30°. The utilization degrees of carbon sources were determined by nephelometry of turbidities resulting from the proliberation of the cells using a Pulfrich photometer. The extinctions per one carbon atom of each carbon source used were calculated from the observed extinctions (E) by a formula : Extinction/carbon atom $=EM/n$ ($M=$molecular weight; $n=$ number of carbon atoms in one molecule) as follows: Sucrose 75.0; glycerol 72.4; D-glucose 72.4; D-mannose 70.8; maltose 70.0; D-fructose 69.2; raffinose 52.0; citric acid 33.6; pyruvic acid 32.2; D-xylose 26.4; succinic acid 25.2; D-mannit 22.4; DL-malic acid 19.0; DL-lactic acid 18.8; malonic acid 17.6; D-sorbit 17.2; ethyl alcohol 16.8; L-arabinose, 15.0; DL-propylene glycol 14.0; propyl alcohol 12.0; propionic aldehyde 11.0; D-galactose 9.2; butyl alcohol 9.2; dulcit 7.8; D-tartaric acid 6.4; L-rhamnose 6.0; acetone 4.6; lactose 3.2; glycol 2.6 Propionic acid methyl alcohol, acetic acid and butyric acid were not utilized. In conclusion, many carbon compounds smaller than sucrose in molecular weight were utilized by *Torula utilis* and the utilization degrees by the yeast depend on the chemical structures of the compounds.

91. Synthesis of flavin compounds by *C. diphtheiae*. By SUSUMU MUTSUHASHI, MASAHIRO MURAKAMI (*From the Institute for Infectious Diseases, Tokyo University, Tokyo*). KUNIO YAGI and ENZO SUZUKI (*the Department of Biochemistry, School of Medicine, Nagoya University, Nagoya*). It was reported that the excess of iron inhibited the toxin formation by *C. diphtheriae*, and high level of the toxin was produced in an iron-deficient medium. It was further suggested that the diphtherial cytochrome b may be the enzyme postulated to contain iron, porphyrin and toxin, and that the diphtheria toxin may be the protein moiety of cytochrome b. For the study of the prolbem that *C. diphtheriae* respires in an iron-deficient medium, *C. dephtheriae* of Park Williams No. 8 strain was cultivated by shaking culture, using Pope's tryptic digest broth as a basal medium, and the synthesized flavin compounds were determined separately by paper chromatography with the simplified lumiflavin method. A high level of flavin compound was synthesized by *C. diphtheriae* when iron was carefully removed from the medium. Total flavin in bacteria after

40 hours' cultivation in an iron-deficient medium was 3.67 per mg. dry weight; that in the medium containing 20γ of iron/ml., was $0.66\,\gamma$/mg. dry weight. The addition of α,α'-sipyridyl or cyanide to the medium promoted the synthesis of flavin compounds by *C. diphtheriae*; the faborable concentrations were $2\text{–}8\times10^{-5}M$ α,α'-dipyridyl, and $2\text{–}4\times10^{-3}M$ cyanide. The flavin compounds contained in the bacterial cells were found to be consisting of FAD and FMN; and in the culture medium filtrate, FMN and free riboflavin were contained. O_2-uptake of *C. diphtheriae* was independent of the iron content in the culture medium. These results suggest that *C. diphtheriae* has a different bypath easy changeable from a main path, such as succino-cytochrome b system, which is proposed by Pappenheimer to be the wide pathway in the oxidation by this bacteria. When the iron system of *C. diphtheriae* becomes decreasingly available, the organism may be adepted to survive by increasing its flavin compound or flavoprotein system.

Naoki Nishida: How high Lf values were obtained in these experiments?

Kunio Yagi: Lf values of the iron deficient media after 40 hours' cultivation were 120, while those of the iron sufficient media were zero.

Makoto Niwa: When iron is decreasingly available, respiration chiefly takes place by flavoprotein and catalase activity also drops, then hydrogen peroxide will accumulate and become poisonous for the respiration.

Kunio Yagi: We proved the catalase activity in the bacteria cultivated in an iron deficient medium, though its activity was comparatively low. Minute iron will be effectively utilized for the formation of catalase.

92. The reduction of nitrates by *Cl. welchii*. By TADAO KATSURA, MASAKO NEMOTO, TOKUKICHI NOJIMA (*From the institute for Infectious Diseases Tokyo University, Tokyo*), SHUSUKE HIRANO (*the College of Medicine, Keio University, Tokyo*), AND FUJIO EGAMI (*the chemical institute, Faculty of Science, Nagoya University, Nagoya*). In order to elucidate the nature of the nitrate activating enzyme and to investigate the role of nitrates in the metabolism of the anaerobic bacteria, the reduction of nitrates was studied by using *Cl. welchii*, one of the strictly anaerobes. The present report deals with the reduction of nitrates to nitites by the washed cell suspension of *Cl. welchii*. The suspension strongly reduces nitrates to nitrites when glucose or ethyl alcohol is used as a hydrogen donator. Studying the initial course of the reaction by using a dilute bacterial suspension, it is possible to follow mainly the reductive reaction, from nitrate to nitrite, neglecting the effect of further reduction of further reduction of nitrite formed. It has been found that the addition of nitrate to culture media causes the increase in the nitrate reducing activity of the cells, but it gives no effect on the dehydrogenase activity. In the coupled reaction with the dehydrogenase system, neutral red can serve as an intermediary hydrogen carrier in the reduction of nitrates by *Cl. welchii*. but this is not the case with the redox indicators of higher potential such as nile blue and methylene blue. KCN, monoiodoacetate, and thiourea strongly inhibit the reduction of nitrate, accompanying no appreciable inhibitory effects on the dehydrogenase activity. NaF and CO have no effect on the reduction of nitrates. These results of the inhibition are similar to those found with the nitrate reductase of *Bac. coli*, except the effect of CO. In manometric experiments, $10^{-3}M$ KCN causes no inhibition on the hydrogenase activity (methylene blue as the acceptor), but almost completely inhibits the activity of the nitrates reduction. These results clearly indicate the presence of the nitrate activating enzyme in *Cl. welchii*, distinct from the dehydrogenase or hydrogenase enzymes. The cells grown on iron-dificient media show weaker dehydrogenase activity and hence lower activity of nitrate reduction.

93. An antibacterial polypeptide crystal isolated from a certain strain of *Bacillus brevis*. By HIROAKI NAGANO and YOSHITAKA SAITO (*From the Department of Biochemistry, Osaka City Medical School, Osaka*). From the culture medium of a certain strain of *Bacillus brevis* from soil, an antibiotically active principle was isolated in crystalline state by the procedure resembling that which Dubos had used to isolate gramicindin. This substance was crystallized from alcohol as colorles, needles, with m.p. 265-267°, and $[\alpha]^{18}_{D}=-272°$. It was practically soulble in alcohol and chloroform. Ornithine, valine, proline, phenylalanine and leucine were identified in its acid hydrolyzate by paper partition chromatography. Strong anitibiotic activity was observed against *Bacillus subtilis*, *Bacillus anthracis*, gram-positive and -negative cocci. By counter-current distribution technique using chloroform, methanol and $0.1N$ HCl as solvent and 140 transfer single-withdrawal method, the above mentioned crystalline active substance was divided in three substances: The substance A, the main part of orginally obtained crystal, melts with decomposition at 272°, and has a high specific rotaion of $[\alpha]^{18}_{D}=-274°$; the elementary analysis and Van Slyke amino N determination coincided with those of gramicidin S; an absorption maximum at 258 mμ in methanol solution of this substance. As to the other two fractions the experiments are now in progress.

94. The amino acids in the hydrolysates of *Streptococcus viridans* and hyaluronidase production by it. By TAKAMI YOSHIDA, HISAYOSHI SUGIHARA, KIMIKO AOKI and TAKAKO SAWAHATA (*From the Kono Clinical Midichine Research Institute, Tokyo*). The strains of *Streptococcus viridans* were detected and isolated from rheumatics and from endocarditis lenta patients. The stains were found to belong to *Str. sanguis*, *Str. mitis*, *Str. bouis*,*Str. faecalis* and *Str. equimus*. They were cultivated for 24 hours in dextrose broth and thioglycolate broth, separately; and cells were collected, hydrolyzed in 20% HCl, and the contents of amino acids were determined by paper partition chromatography. Leucine, methionine, valine, α-alanine, threonine, serine, glutamic and aspartic acids, cystine, arginine and histidine were detected in all strains; the variation of the culture media gave no effect on the kinds of amino acids detected. The contents of leucine, glutamic and aspartic acids were variable with the type of *Streptococcus*. The production of hyaluronidase was observed in *Str. bovis* (S) cultivated in dextrose broth and in *Str. bovis* (F) grown in broth alone. The maximum production was found on the 3rd day by S-strain and on the 2nd day by F-strain. Sensitivity of those strains to antibiotics was calculated by dilution method. The sensitivity of each strain to penicillin, streptomycin, aureomyocin, chloromycetin and supronal were not corresponding to the type of the strain. No interrelation has been found among amino acid composition, hyaluronidase production and sensitivity to antibiotics with 5 strains of *Str. viridans*.

95. Studies on the dissociation of L-histidine. The dissociation of DL-formylisoglutbmine by resting *B. subtilis*. By YOSHITO NISHIZAWA, TEISUKE KODAMA, YOSHIKAZU OZAWA AND YASUO MORIMOTO (*From the Department of Pediatrics, Osaka University, Osaka*). L-Isoglutamine is an itermediary metabolite of L-histidine, but there has been some question as to formylisoglutamine which is believed to be its precursor. By the action of the resting cells of B. *subtilis* on DL-formylisoglutamine, both L-glutaminic acid and undissociated DL-formylisoglutamine were obtained. Though formylisoglutamine is believed to be an intermediary metabolite of L-histidine, the problem of why it takes the racemic form is left for further study.

96. A study on intermediary metabolism of histidine. By MANJI INOUE (*From the Institute of Medical Chemistry, Nagasaki University Medical School, Nagasaki*). The detection of imidazole derivatives in urine was carried out by paper partition chromatography after the administration of histidine to fasting rabbits. Histidine and urocanic acid were usually found in normal human and rabbit urine. Besides them, some new intermediates were detected in rabbit urine: the one was identified as a imidazole derivative, and the other was likely to be imidazolic acid. Starved rabbits seemed to excreted more urocanic acid than normal ones. The enzyme active for histidine was prepared from rat liver extracts by the Edbacher's method (A-extract) and by the Blanchard's method (B-extract). After incubation histidine with the A-extract at pH 8.2 (phosphate buffer), only urocanic acid was detected as the end-product; while B-extract yielded imidazole acetic acid from histidine and also an unidentified imidazol derivative besides urcanic acid. Thus two histidine-decomposing enzymes have been demonstrated in rat liver: the one disintergrates histidine into urocanic acid, and the other, possibly through imidazol pyruvic acid, converts histidine into imidazole acetic acid. The following imidazole derivatives were used in the experiments: Histidine. HCl, histamine. HDl, imidazol acetic acid, imidazole lactic acid, imidazole propionic acid, urocanic acid, and imidazole carbonic acid. Their respective Rf-values were determined by paper partition chromatography by use of butanol-pyridine-water (1:1:1), or butanol-acetric acid-acetic ether-water (1:1:) as a developing solvent.

97. Studies on the metabolism of histidine. By KIYOSHI MATSUDA, JUYO ITAGAKI, TSUTOMU WACHI AND MAKIO UCHIDA (*From the Department of Biochemistry, Osaka University Medical School, Osaka*). *Histidine-deaminase* The enzyme was extracted from the rabbit liver fortified by the administration of histidine, and was purified by isoelectric precipitation, fractionation with $(NH_4)_2SO_4$, and adsorption on Ca-gel, seccessively. The enzyme fractionated at pH 5.2 was activated to 95% of the original by the addition of folic acid. The dialyzed preparation was also reactivated by the addition of either folic acid or glutathione, and completely by adding folic acid plus glutathione. The enzyme prepared from the rabbit liver pretreated with aminopterin had a fairly low activity, which was more markedly restored by the addition of folic acid than the enzyme from normal rabbit liver. The adsorption of the enzyme fraction with 30 to 50% $(NH_4)_2SO_4$ on Ca-gel resulted in a complete resolution of the enzyme; the resolved purified enzyme restored its activity to 80% by the adition of folic acid, and completely by the simultaneous addition of folic acid and glutathione; but glutathione alone had no effect. The concentration of folic acid (estimated microbiologically) required for the maximum restoration of the resolved enzyme activity showed a saturation level of 200γ. The existence of urocanic acid was demonstrated by means of paper chromatography in the reaction mixture composed of the resolved purified enzyme, folic acid and glutathione. From the results obtained, it has been confirmed that folic acid acts as the coenzyme of histidine-deaminase in the presence of glutathione. *Urocanicase*: The conversion of urocanic acid, hydantoin-propionic acid or carbamyl glutamic acid into glutamic acid by crude urocanicase were quantitatively demonstrated by using glutamic acid decarboxylase prepared from squashes. The O_2-uptake in the complete decomposition of urocanic acid by the purified urocanicase was decreased by 70% by the addition of α-ketoglutaric acid. The enzyme fractionated with $(NH_4)_2SO_4$ after adsorption on Ca-gel degraded urocanic acid anaerobically; while when pyruvic acid was added, the complete decomposition of urocanic acid was observed, where the O_2-uptake was 80% of its theoretical value for its decomposition. From these results obtained it has been suggested that urocanic acid is decomposed by way of hydantoin-propionic acid; and that a dismutation conceivably takes place between 5-hydroxyimidazolyl-propionic acid and α-ketoglutaric acid, which is inhibited by pyruvic acid.

Akira Kato: Is the enzyme which catalyzes the reduction of α-ketoglutaric acid contained in the same preparation? Did you estimate the amount of the decrease in α-ketoglutaric acid?

Makio Uchida: Further investigation for identifying the enzyme concerned with the dismutation and the estimation of α-ketoglutaric acid is now being carried out.

Akira Kato: In considering the anaerobic conversion of urocanic acid into glutamic acid, is there any possiblity of that hydantoin-propionic acid is a metabolite?

Makio Uchida: As to the dismutation concerning 5-hydroxoimidazolyl-propionic acid, the anaerobic decomposition of urocanic acid is compatible with the behavior of hydantoin-poopionic acid as one of its metabolites.

Akira Kato: What do you think of a possiblity of the existence of a certain liver enzyme responsible for hydantoin-propionic acid decomposition, which is independent of urocanicase system?

Makio Uchida: From the data confirming the behavior of hydantoin-propionic acid as one of the metabolites, the enzyme concerned is considered to be related to this system.

98. Studies on the metabolism of tyrosine. By SHUSAKU SUZUKI, ICHIRO UEMATSU AND MAKIO UCHIDA (*From the Department of Biochemistry, Osaka University Medical School, Osaka*). The mechanism of the oxidative degradation of *p*-hydroxyphenylpyruvic acid *via* homogentisic acid has been investigated, *The Conversion of p-Hydroxyphenylpyruvic Acid to Homogentisic Acid*: The *p*-hydroxyphenyl-pyruvate—oxidizing enzyme system was extracted from rabbit liver, and purified by fractionation with $(NH_4)_2SO_4$ and adsorption on Ca-gel. The enzyme preparation of 30 to 50% fraction with $(NH_4)_2SO_4$ showed the highest activity. The enzyme was separated from the homogentisic acid-oxidizing enzyme system by inactivating the latter at pH 5.0. Thus prepared pure enzyme was completely inhibited by oxine or H_2S, inhibited up to 80% by NaN_3 and partially by Fe^{++} α,α'-dipyridyl, but CO showed no inhibition. The inactivated enzyme which had not been subjected to isoelectric treatment restored its activity by the addition of vitamin B_{12} and vitamin C, accompanying a marked production of dihydroxyphenyl compounds, but no acetoacetic acid was detectable. The inactivated enzyme also regained its activity by the simultaneous addition of vitamin B_{12}, vitamin C, DPN, co-carboxylase, adenylic acid and glutathione, the latter four compounds, combined or alone, had no effect on the restoration. In the presence of the above six compounds the formation of acetoacetic acid and CO_2 were demonstrated. *The Counversion of Homogentisic Acid to Acetoacetic Acid*: The homogentisic acid—oxidizing enzyme system was prepared from rabbit liver by the above procedure. The enzyme activity was greatly reduced by aging for 48 hours (80—100%). But the lost activity could be restored by the addition of either $10^{-3}M$ Fe^{++} or folic acid plus adenylic acid, yielding the theoretical amount of acetoacetic acid. The enzyme was completely inhibited by α,α'-dipyridyl, NaN_3, oxine, Ag^+ or Hg^{++}; partially by monoiodoacetic acid or Ca^{++}; but not by KCN. The aged enzyme for 24 eours was sensitive to KCN. Methylene blue did not serve as a hydrogen acceptor in the initial stage of homogentistic acid oxidation. The NaN_2-inactivated enzyme restored its activity by the addition of either Fe^{++} or $Na_2S_2O_4$. The enzyme preparation obtained by elution after Ca-gel adsorption was completely inactivated. The resolved enzyme was not reactivated by the addition of the combination of folic acid, glutathione, adenylic acid, and vitamin C; but it can be restored by the further addition of either DPN or vitamin B_{12}, and most effectively by the addition of DPN plus vitamin B_{12} to the above 4 compounds. Either Fe^{++}or Fe^{+++} plus vitamin C or each one of the 6 compounds alone had no reactivating effect. In view of the results obtained, vitamin B_{12} is operative in the presence of vitamin C for the *p*-hydroxyphenyl-pyruvic acid-oxidizing enzyme system, and also takes a part in the enzyme system responsible for homogentisic acid oxidation in the combination of vitamin C, DPN, folic acid, glutathione and adenylic acid.

99. The effect of experimental ketosis on tyrosine metabolism. By YOSHIRO TAKEDA, KIYOSHI SUJISHI AND MASARU HARA (*From the Department of Bacteriochemistry, Research Insusite for Microbial Diseases, Osaka University, Osaka*). In the course of the purification of an enzyme system catalyzing the breakdown of *p*-hydroxyphenylpyruvate to homogentisicate, it has been found that this enzyme ststem is strongly inhibited by keto acids such as pyruvate. Therefore, it is believed that some interaction might exist between tyrosine metabolism and carbohydrate or fatty acid metabolism. The administration of a large amount of sodium butyrate to guinea pigs along with L-tyrosine resulted in the urinary excretion of phenolic compounds, upon which L-ascorbic acid had no effect. The phenolic compounds excreted in urine were found to be consisting of a predominating amount of *p*-hydroxyphenylpyruvate and a small amount of 2,5-dihydroxyphenylpyruvate; but no homogentisicate was entirely detectable. The excretion of phenolic compounds in ketotic alcaptonuria ceased upon the administration of malate. The urinary excretion of phenolic compounds in ketotic alcaptonuria reached the maximum value in 4–5 days after the start of experiment, and was rapidly lessened thereafter. When pyridoxine was administered at the peak of phenolic compounds excretion, the excretion of phenolic compound did not decrease but it was rather sustained at a constant level. When pyridoxine was administrated at the decreasing stage of the excetion, the phenolic compound excretion increased again.

100. Metabolism of L-tyrosine by *Streptomyces phaeochromogenus*. By YASUYUKI TAKAGI AND KAHEI OKUNO (*From the Department of Biochemistry, Osaka City Medical School, Osaka*). A strain of chromogenus type of *Streptomyces phaeochromogenus* was isolated from soil, and cultivated as a slant culture on the mineral medium of Czapecka and Dox supplemented with glycerol and agar. The basal medium used for assay was the same, but without agar. On this medium the bacterial growth was so slow that yeast extract was added as a vitamin-mixture to this basal medium. The material to be tested was added at a desired concentration to 5 ml. of the basal medium with the double strength in a 30×200 mm.-test tube. The culture was then diluted to 10 ml., and incubated at 30° for 36 hours. The culture on the basal medium produced not pigment even after 7 days. Among various types of inorganic and organic nitrogenous compounds tested instead of sodium nitrate, only the addition of tyrosine was effective to the pigment formation; and phenylalanine was slightly effective. The similar effect was also brought about by tyrosine-hydantoin, tyramine, or benzoyl-tyrosine, but not by *o*-tyrosine diiodotyrosine, tyrosine-sulfonic acid and *p*-hydroxyphenylpyruvic acid. Many carbohydrates were tested as the C-source but they showed the essential effects on the bacterial growth, but gave no direct effect oh the pigment formation. A marked pigment formation occurred only in the co-existence of tyrosine and yeast extract, suggesting a factor necessary for pigment formation from tyrosine. This factor could be obtained from yeast, liver, kidney, corn steep, liquor, soy bean meal *etc.*; but the yeast factor was the most active. The yeast factor was soluble in water, but almost insoluble in various organic solevents, and was stable against hydrolysis with 1 *N* H_2SO_2 or 1 *N* NaOH at 120° for 15–30 minutes. Heating with 4 *N* acid for 30 minutes caused only a slight loss in its activity; the active material was precipitated with mercuric, silver, and tungstic ions, and was adsorbed on Fuller's earth and charcoal. Adsorption on Fuller's earth followed by elution with baryt, or treatment with charcoal followed by elution with ammoniacal alcohol gave good recoveries of the factor. This active factor was not identical with any one of known vitamins. The purification of the active factor has been tried by the following procedures: Acid hydrolysis with 1 *N* H_2SO_4 for 30 minutes at 15 pounds pressure in an autoklave; adsorption on Fullers' earth followed by elution with baryt; treatment with charcoal followed by elution with alcoholic ammonia; (d) precipitation with silver ions at pH 2–8.5; and treatment with tungstic ions. Final fraction had the activity to increase markedly the pigment formation without effecting the bacterial growth; but a greater part of the activity was always lost during these procedures. The attempts of further purification so far has been unsuccessful. Thio factor had similarity in some respects to pyruvate oxidation factor of O' Kane and Gunsalus. Cystine, diethyldithiocarbamate, sodium thiosul fate and furacin showed the inhibiting effects on the pigment formation at a concentration, exhibiting no effect on the bacterial growth. *o*-Tyrosine and diiodotyrosine gave no effect on the pigment formation. The results of inhibition may indicate that the copper protein plays a direct rôle in this process of pigment formation, as in case of melanin formation. It can be further suggested that the pigment produced by the strain of *Streptomyces sp.* may be melanin itself or, at least, melanin-like substance. The oxidative pathway of tyrosine by this strain was analyzed by use of the successive adaptation method: *p*-Hydroxyphenylpyruvic acid and 2,5-dihydroxyphenylpyruvic acid were oxidized by the cells adapted to tyrosine; whereas homogentisic acid was not metabolized by them. It is most hrobable that tyrosine is oxidized in case of this microorganism *via* the following sequence of reactions: Tyrosine ⟶ *p*-hydroxyphenylpyruvic acid → 2,5-dihydroxyphenylpyruvic acid ⟶ $CO_2 + H_2O$.

101. On the fluctuation of methionine content at hatching eggs. By SOTEN TAKAHASHI (*From the Department of Medical Chemistry, Osaka Medical College, Osaka*).

102. Photometric estimation of hinokitiol, an antibotic extracted from *Chamaecyparis taiwanensis Masamune et Suzuki*. By ICHIRO TERADA (*From the Department of Medicine, Niigata University School, Niigata*). To estimate the concentration of hinokitol photometrically, ferric chloride was used as a color reagent. This method, however, was proved to have various disadvantages for assay of the antibiotic contained in the clinical specimens. The author has established an improved photometric method using ferric ammonium citrate as a reagent, which develops greenish color and enables us to detect the antibiotic being contained only a few mg. per ml. of the urine. Using this method, it has been found that hinokitiol excreted in urine consists of two fractions, *i.e.*, free and conbined forms. The latter form can be fractionated in two parts: the one develops characteristic color with the reagent, but the other develops the color only after hydrolysis with hydrochloric acid.

103. Anthranilic acid in urine. By YOICHI SHIRAI, SEIKI TANI, MITSUO OKADA AND TAKAO SASAOKA (*From the Department of Biochemistry, Wakayama Medical College, Wakayama*). There is no doubt about the production of anthranilic acid from tryptophan as a metabolite. But it has never been observed that anthranilic acid is produced directly from tryptophan in mammals nor that anthranilic acid exists in normal urine. Consequently the breadth of the following pathway remains to be determined: Tryptophan ⟶

kynurenine ⟶ anthranilic acid. To clarify this problem, urinary excretion of anthranilic acid was examined after the administration of the acid. Anthranilic acid did not exist as free but almost completely conjugated with glucuronic acid. The type of this glucuronide is the ester-type, as Prof. Ichihara claimed 25 years ago; in other words, carboxyl group of anthranilic acid conjugates with the acetal-OH of glucuronic acid. This conception is now ascertained chiefly by the observations that the both components of this glucuronide are readily split off from each other by gently warming at the alkalinity of *N*/10-NaOH. Since the anthranilic acid glucuronide is hardly soluble in ether it is necessary to hydrolyze the urine prior to anthranilic acid detection. The urine of rabbit administered with tryptophan subcutaneously was hydrolyzed with 1 *N*-HCl and extracted with ether. From the paperchromatographical results, the ether-soluble fraction contained anthranilic acid together with three other unidentified aromatic amines which are considered to be tryptophan metabolites. From the density of fluorescence, the exeretion of anthranilic acid was calculated to be about 30 mg. per g. tryptophan administered. Thereafter, the authors have been able to detect anthranilic acid in normal human urine by the method described above. The concentration of anthranilic acid in human urine was 0.5 mg. per liter on the average.

Katashi Ichihara: The excretion of ethereal sulfate and conjugated glucuronic acid was increased in the rabbit urine by adminstering anthranilic acid-hydantoin. 5-Hydroxyanthranilic acid-hydantoin was demonstrated from the hydrolyzate of its glucuronide by means of paper-chromatography. From our observation, it is considered that authranilic acid-hydantoin is oxidized *in vivo* to 5-hydroxyanthranilic acid-hydantoin and that either its glucuronide or ethereal sulfate might be formed through its hydroxy-group.

104. Occurence of diabetic symptoms caused by an abnormal metabolite of tryptophan. By YAHITO KOTAKE AND TOSHIRO INADA (*From the Biochemical Institute, Wakayama Medical College, Wakayama*). Administering sodium butyrate to animal, it was found that the acute vitamin B_6-deficiency occured. When butyrate was administered together with tryptophan, the animal discharged a large amount of xanthurenic acid, which was isolated in crystalline form (m.p., 290°). Noteworthy was the fact that the diabetic symptoms were observed in the animal when injected 120 mg. of xanthurenic acid per kg. body weight. Uprisings of blood and urine sugar, selective invasion of the beta-cells the Langerhan's islets of the pancreas, eye cataract *etc.* were noted. During the course of this study the existence of an enzyme in the rabbit liver was considered to decompose xanthurenic acid.

105· Biochmical studies on the lysine derivatives. By TOSHIO YONEYA (*From the Department of Medical Chemistry, Faculty of Medicine, Universiy of Kyoto, Kyoto*). Of the acyl derivatives of DL-lysine only ε-benzoyl-α-chloroacetyl-DL-lysine was split to 50% by the hog kidney enzyme. After the incubation of the α,ε-diacyl-DL-lysine at 37° for 17 hours with the aqueous extract of the acetone-dried hog kidney powder, ε-benzoyl-L-lysine (m.p., 267-268°; $[\alpha]_D^{20} = +17.1°$) was separated from the acidified filtrate by the addition of absolute alcohol. L-Lysine dihydrochloride was obtained by the acid drolysis of the benzoyl compound. The mother liquor obtained from the precipitate of the ε-benzoyl-L-lysine was extracted with ethyl acetate, which was evaporated to dryness. From the hydrolyzate of the residual syrup, D-lysine dihydrochloride was isolated. Thus DL-lysine was favorable resolved by means of the asymmetric enzymatic hydrolysis with the hog kidney enzyme. If the ε-amino group of the lysine is to be unmasked in the protein, its ε-peptide will be probably resistant to the proteolytic enzyme. From the view point of specificity, the susceptibility of ε-gylcylaminocaproic acid and ε-benzoyl-α-glycyl-DL-lysine for the hog kidney and beef liver enzymes was tested. Both enzymes attacked the compounds (100% splitting for the former, 50% for the latter). Contrary to the enzymatic resistance of the acyl derivatives of the ε-aminocaproic acid, ε-peptide such as ε-glycylaminocaproic acid, was completely hydrolyzed. The acylase action is presumably different from the dipeptidase one.

106. Effect of various amino acids upon the distribution of porphyrin bodies in tissues. II. Effect of sulfur containing amino acids. By TAKEKAZU KOSAKI AND TADAO IKEDA (*From the Biochemical Institute, Mie Medical College, Mie*). The contents of porphyrin bodies in various tissues of guinea pigs administered with cytine, cysteine or methionine by subcutaneous injection have been measured by the method of Kosaki. The changes in the porphyrin bodies content of tissues are most marked and rapid with cysteine, and least marked and slow with methionine. Cystine when given in its excess dose or given repeatedly in its small doses causes the decrease in the metalloporphyrin content of liver, *etc.* The most conspicuous effect is found to be exhibited at an optimal concentration of each amino acid. (*cf. J. Biochem.*, **39**, 367 (1952)).

107. Effect of various amino acids upon the distribution of porphyrin bodies in tissues. III. Effect of ε-amino caproic acid and related substances. By TAKEKAZU KOSAKI, TADAO IKEDA AND YASUMICHI NODA (*From the Biochemical Institute, Mie Medical College, Mie*). ε-Amino caproic acid is known to exert a marked physiological action. The effect of caproic acid, ε-amino caproic acid, norleucine, and lysine on the distribution of prophyrin bodies in tissues of guinea pigs has been investigated. Most marked is the effect of ε-amino caproic acid, increasing the metalloporphyrin of various tissues. The other acids exert the similar action, the acitivity of which seems to be dependent on the position of the amino group substituted. (*cf. J. Biochem.*, **39**, 381 (1952)).

108. A contribution to the acid hydrolysis of protein. By YOSHIHISA TOMOHIRO, KENJIRO AZUMA, YOSHIO HIKITA AND GENJI MATSUDA (*From the Department of Biochemistry, Nagasaki University Medical School, Nagasaki*).

109. Ogawa's colloid reaction as an index available for the observation of the process of protein hydrolysis. By IWAO OGAWA, TATSUTO SHIMIZU, YURIKO YOKOO (*From the Research Institute of Environmental Medicine, Nagoya University, Nagoya*), AND ICHIRO ISHIHARA (*the 1st Department of*

Internal Medicine, Nagoya University School of Medicine, Nagoya). Ogawa's colloid reaction, of urinary colloid as an index of the process of protein hydrolysisis is also clinically useful to detect the severity of protein catabolism in cases of pulmonary tuberculosis or " alarm reaction." A remarkable finding in the graphical diagram is the characteristic " extention," namely, the phenomenon that the same color of Ogawa's colloid reaction remains unaltered in spite of dilution; the " extention " of Y is produced by peptone; and that on and around YR by proteins. In the process of protein hydrolysis, the initial " extention " on YR is destructed and then the " extention " of Y appears. Thereafter, the latter is prolonged increasingly to the maximum. Then it shortenes and finally disappears. This may suggest that the original protein particles are hydrolyzed to the smaller molecules such as metaprotein, proteose, peptone *etc.*, and finally almost to amino acids, that is also confirmed by the amino acid pattern of each hydrolyzates on paper chromatogram. This reaction has been applied as clinically an index to protein catabolism The " extention " on Y is very prolonged, that is, the value of protective by numerical method is very high in the urine of far advanced cases of pulmonary tuberculosis. In the stress, such as surgical operotions, there occurs the prolongation of the " extention " on Y along with the cosinopenic response. This is thus encluded to be a probable indicator to " alarm reaction."

Atsushi Oshikane: An odd-looking phenomenon, under mentioned, was found in the case of hydrolysis of human saliva by boiling with HCl, and we consider that this phenomenon may be useful as an interesting mark in the study of the hydrolytic process of some molecular compounds. The phenomenon is that the foaminess of saliva is soon lost by boiling with concentrated HCl, but, by continuation of boiling thereafter, the foaminess of the liquid comes back again slowly after about 120 minutes; the foaminess becomes higher by and by, and the points of remarkable enlargement of the foam stability (duration of the foam layer) appear with some periodicity. We cannot clarifty the reason or the essence of this phenomenon up to date.

110. A study on protein denaturation. By SHIMPEI ARAYA (*From the Department of Biochimistry, Tokyo Medico Dental University, Tokyo*). It has been reported by the present author that the weak alkaline biuret reaction represents very delicately the denatured state of the protein. This method can be quantitatively carriod out as follows: An excess of $Cu(OH)_2$ is added to the sample solution at a definite pH as low as possible (pH 8-9), the solution is centrifuged and the supernantat is colorimetrically measured. The present report first deals with the enzyme in *Ricinus communis* which makes the protein more reactive in the weak alkaline biuret reaction. This enzyme is thermolabil and non-dialyzable. The optimum pH is 4.8, and the optimum temperature, 45-50°. In the biuret reaction of egg albumin the enzyme makes it more reactive without increasing the values of the formol titration. In this case, therefore, the hydrolysis of the peptide bonds may be out of the question. Where as in the horse serum euglobulin, the value of the formol titration increases slightly with the rise of the biuret reaction reactivity. In this case, however, no effect is found by adding monoiodacetate. The enzyme cannot make the egg albumin reactive in the nitroprusside reaction. It seems, therefore, that the enzyme gives a mild modification of the protein configuration. The second problem of this report is as follows: When the egg albumin solution is denatured by heat, the reactivity of the ninhydrin reaction decreases gradually with time. The reactivity immediately after the denaturation, is the same as that of the native protein. Therefore, this phenomenon does not deal with the regeneration of the denaturation. It seems that this may deal with an intermolecular aggregation or a new intramolecular folding of the peptide chains.

Tetsuo Hayashi: As I have reported, iodoacetate or any of the alkylating reagents have in fact so poor affinity to -SH groups, that I recommend you to employ more specific and more powerful regents to -SH groups; for example, *p*-chloromercuric benzoate.

111. On the protein-dissolving action of homosulfamine, *p*-aminosaliclic acid and other agents. By MOTOYUKI YOSHINARI (*From the 1st Surgical Clinic, Faculty of Medicine, Kyushu University, Fukuoka*). The discovery of the profibrin-dissolving action of homosulfamine led the author ot another discovery that the aqueous solution of para-aminosalicylic acid, sulfaisoxazole, acetosulfamine *etc.* had a protein-peptizing action. This action has been studied in comparison with the similar action of urea. The action by which powdered protein is dissolved or by which aggregated protein is peptized, is considered the in respects to the time needed for complete dissolution. The turbidity is determined by a nephelometer, and the total N by the modified Parnas's method. The conclusion is as follows: Homosulfamine, PAS, sulfaisoxazole *etc.* far more strongly dissolve or/and disperse proteins that urea does; they also readily dissolve protein even at isoelectric point, but they can not dissolve denatured proteins; they can dissolve pyoteins completely, while urea dissolution is accompanied by partial aggregation after denaturation; at higher concentrations, they dissolve starch as well as proteins.

Hidematsu Hirai: Have the drugs a power of dissolving bacteria, leucocytes, *etc.* from tangible into intangible forms? Is the process microscopically observable?

Motoyuki Yoshinari: The pus is so uniformly dispersed in the aqueous solution of the drugs that nothing concrete remains macroscopically detectable. No microscopical examination has yet been attempted, but a report will be presented on the matter at no distant future.

Hidematsu Hirai: The data presented do not appear sufficient to establish the difference between the action of the drugs and urea. The fact that urea accompanies the dissolution and aggregation of proteins, while the drugs are by its dissoltion alone, seems to require further physicochemical investigation for explanation.

Motoyuki Yoshinari: I highly appreciate your pertinent advice. A more detailed explanation will be given in the forthcoming report.

112. Attitude of serum protein and dental pulp protein in the case of experimental maxillary osteomyelitis. By ATSUSHI OSHIKANE AND GAKUJI SATO (*From the Biochemical Department, Dental School, Nihon University, Tokyo*). The authors have succeeded in producing osteomyelitic experimentally in the mandibular bone of a young dog by perforation into the bone, charge with electric current, and infuison of suspension of *Staph. aureus* into the marrow. In the period of acute inflammation there were observed the decreases in total protein, albumin, albumin-globulin ratio and γ-globulin, and rather the increases in total globulin, α-globulin and β-globulin. In the period of chronic inflammation, total protein, albumin, albumin-globulin ratio, β-globulin and γ-globulin increased, but total globulin and α-globulin were rather lowered. In the extract from the pulp, proline and aspartic acid appeared, and β-alanine and glutamin disappeared in the period of chronic osteomyelitis. As to the residual substance of the pulp, alanine appeared in the period of chronic osteomyelitis.

113. Studies on protein metabolism in the muscular exercises. By HISATO YOSHIMURA and SHUNICHI USAMI (*From the Department of Physiology, Kyoto Prefecturral Medical College, Kyoto*). Yamaji reported the increase of non protein nitrgen (N.P.N.) in blood, owing to promotion of the protein catabolism during heavy muscular exerise. The author studied the problem under various conditions of the subjects, and of load, and with the blood samples taken from various part of the body. Results obtained are as follows: (1) The more intense the muscular exercise is, the higher is the rise of N.P.N. level in the blood; (2) in the muscular exercise after fasting or injection of cortisone (100 mg), N.P.N. level rises more highly; (3) the rese of N.P.N. rather decreases after intake of sugar or injection of adrenalin or during the inhalation of oxygen; (4) the value of N.P.N. of the venous blood collected from contracting muscles is higher than that from the resting one. It is concluded that the catabolism of protein is accerelated in the muscle during its exercise, and is related with energy supply to the muscle.

114. Studies on tissue proteins. IV. Extraction of tissue proteins by successive extraction. By SHOJIRO NAKAMURA, MOTOYUKI TAKAHASHI, TOMOHARU ZAIZEN, AND KAZUSHIGE TANAKA (*From the Department of Medical Chemistry, Yamaguchi Medical College, Ube*). When the tissue brei is extracted successively with an equal quantity of physiological saline solution, the extraction rate, *i.e.*, the rate of the protein concentrations of the successive extracts, K′, will be: $K'=C_n/C_{n-1}=1/(1+\rho_{n-1})$, ($C_n$ is the protein concentration of the n'th extract, and ρ_n is the density of the n'th precipitate). This relationship can be confirmed in cases of the extraction of liver, kidncy, lung, spleen, and muscle, but not with intestinal mucosa. It is doubtful whether the incompatibility depends upon the partition of proteins between the extract and the precipitate. The relative concentrations of the electrophoretic fractions of each successive extract were nearly constant with liver and kidney, but somewhat changed with intestinal mucosa. Conditions of extraction, *i.e.*, pH and concentration of salt solution, that could affect the extraction rate, K′, were studied with liver, and some suggestive results were obtained.

115. Studies on tissue proteins. V. Extraction of the proteins of brain by successive extraction. By YASUSHI HAYASHI (*From the Department of Medical Chemistry, Yamaguchi Medical College, Ube*). When the brain brei is extracted successively with an equal quantity of physioloical saline solution, the obtained extraction rate, K′, was 0.35–0.45. It is definitely smaller than the value, 0.49, calculated from the density, 1.02, of the brei. The reason for this incompatibility seems to suggest that the volume of insoluble materal can not be neglected. This volume estimated by the addition of KI was 20–30% of the original homogenate. (*p*-Nitophenol was adsorbed by the insoluble material, and the adsorption occurred according to the Langmuir isotherm). Relative concentrations of the electrophoretic fractions of each successive extract were nearly constant. Therefore, the obtained value of extraction rate can reasonably be explained by the relative volume of insoluble material. When the homogenate of brain mixed with an equal quantity of saline solution was centrifuged (3500 r.p.m.), the volume of the precipitate was about 20% larger than that of the original homogenate, probably because the originally arranged, insoluble material became particulate by the homogenization.

K. Umeno: I think the attention must be taken of the effects of the solvent, in speaking the solubility of proteins. Yamamoto (*Dept. of Gyn., Nagoya Univ.*) has extracted and analyzed the proteins of placenta electrophoretically, taking advantage of the superiority of 0.2 *M* phosphate buffer at pH 7.8 containing NaCl at a concentration of 1 *M*.

A. Okamoto: A fairly large portion of tissue proteins can only be extracted with alkaline solution or with potassium salt solution of high concentration, but it is insoluble in the physiological saline solution (so-called construction protein). Accordingly, your insoluble material seems to contain a large amount of proteins. It would be preferable to extract with alkali or salt solution of high ionic concentration.

Shojiro Nakamura: It is well known as you say, that the kind, pH and ionic strength of the solvent affect the solubility of proteins. The total quantity of the proteins extracted finally, however, can not be infered only from the solubility (or salting-in effect), as can be seen from our results. In the present experiments, we did not atempt to extract all the proteins contained in the tissues, but to study the extractability of the proteins under our experimental conditions. The insoluble material is always related to the conditions of solvents. The quantities of insoluble nitrogen in the physiological saline solution were not so large, except muscle and a few other organs; for example, lung and brain.

116. On the relation between hemoglobin formation and phosphorus metabolism by method of bone marrow culture. By GORO KOIKE AND YUKIKO SAKAI (*From the Women's Funior College of Nutrition, Tokyo*). Hemoglobin formation has been studied by a modified method of bone marrow culture of Osgood and Brownlee Hemoglobin is determined spectrophotometrically at 541 and 560 mμ. Hemoglobin in the medium increases in 30 minutes, and then gradually decreases. By cultivating the rabbit bone marrow under the same condition, phosphorus compounds are seperated by the fractionation method of Schneider and Schmidt-Thanhauser. Phosphorus content is determined by the modified method of Fiske-Subbarow. Acid soluble organic phosphorus and RNA-phosphorus increases in 30 minutes. Lipid-phosphorus decreases contraversely.

117. Studies in the quantities of

phosphate compounds in fish " chiai muscle " during the growth of the fishes. By KUNIO TERASHIMA (*From the Department of Biochemistry, Iwate Medical College, Morioka*). The " chiai muscle " is furnished with the same function as the mammalian " red muscle," showing a vigorous oxidation. According to the experiments of Obara, the energetic tissue respiration suggests the increasing oxidation in " chiai muscle." This report concerns the quantaties of phosphate compounds contained in the "chiai muscles" of the fish in due course of the growth. The concentration of each phosphate compound fraction in the " chiai muscle " and also in the other muscles decreases in parallel with the development. In further growth, the concentrations are rather invariable. These results suggest that the " chiai muscle " may supply a great deal of energy to the resting muscle for the contraction.

118. Phosphate matabolims of retina cells. II By SHINYICHI TANAKA (*From the Department of Biochemistry, Nagoya University School of Medicine, Nagoya*) In the previous report, the author has proposed a hypothesis on vision that the energy of light caught in retina cell is kindled to the energy rich phosphate compound such as ATP, and then various enzyme reactions take place suddenly. The problem has been supplemented by using isotope P^{32}. The phosphate esterification in retina increases by illumination; especially the energy efficiency of aerobic phosphorylation reaction is markedly promoted in lightened retina. Previously the author has reported that malic acid is oxidized in the presence of DPN, FAD and ATP despite of cyanide inhibition. The fact that the disappearance of orthophosphate and the maintenence of labile phosphate bond suggest that energy efficiency of the reaction caused by such an autoxidizable flavoprotein is not good. In this report it has been clarified that TTP (thiamine triphosphate) accelerates the pyruvate oxidation of retina cell homogenata in the presence of ATP, DPN, niacin amide, cytochrome c, Mg and catalytic amount of C_4-dicarboxylic acid. It has been also found that TTP promotes the syntheses of adenylpyrophosphate and fructose 1,6-diphosphate by use of acetone-dried powder of retina cells. TTP remarkbly activates acetyl choline esterase in retina, but ATP does not; the activation by TTP is not influenced by fluoride.

Norio Shimazono: In our laboratory it has been found that α-ketoglutarate oxidation by heart of thiamine deficient rat is promoted by the addition of cocarboxylase and ATP, but not in the presence of thiamine triphosphate (TTP)

Shinyichi Tanaka: Using Summerson's reaction mixture, containing ATP, DPN, niacin, amide, cytochrome c, Mg, pyruvic acid and a catalytic amount of fumaric acid, the influence of TTP on the pyruvate oxidation of retina cell homogenate was investigated. As the thiamine content of retina cells is much less (160 $\gamma\%$), the above mentioned results may have been obtained in these experiments.

Takamitsu Sekine: I think that from the viewpoint of the enzyme properties the activation of acetyl choline esterase by TTP should be determined by the disappearance of acetyl choline.

Shinyichi Tanaka: There exists no specific method for the determination of acetyl choline. I have estimated the formation of an acid manometrically. Nevertheless, there occurs no acid formation in the presence of TTP only. Therefore, it may be suggested that acetyl choline phosphorolysis will occur.

Takamitsu Sekine: There is no experimental fact that the acetylation of choline may induce the excitation of nervous tissue. Nevertheless it is known that an acetyl choline-lipoprotein may release acetyl choline by the action of a certain stimulus.

Shinyichi Tanaka: It may be suspected that there exists an unknown intimate relation among nervous excitation, thiamine and acetyl choline.

119. The study of the metabolism of acid-soluble phosphate compounds of the rat liver using radioactive phosphorus (P^{32}). By KIKUO OGATA AND CHIKAKU ENOKI (*From the Department of Biochemistry, Niigata University School of Medicine, Niigata.*) The phosphate compounds of acid soluble fractions of rat liver were fractionated one hour after the intraperitonal injection of 15 Curie of radioactive P^{32} as Na_2HPO_4 per 100 g. body weight, and the renewal rate of phosphate of these compounds was compared in the cases of normal, thiamine-deficient and pair-fed control rats. For the fractionation of acid-soluble phosphate compounds we employed principally the method of Kaplan and Greenberg; but ATP and ADP fraction was separated as Hg-salt from Ba-insoluble fraction. For the measurment of the radioactivity of acid-soluble compounds the method of Lehninger was emplyed, but the coprecipitation of ATP with $MgNH_4OH$ was avoided by separating the ATP- and ADP-fractions as described above and by using one-fourth amount of carrier phosphate described by Lehninger. The following acid-solble fractions were obtained and analyzed: (i) Labile P of ATP and ADP fraction; (ii) inorganic phosphate, from Ba-insoluble fraction; (iii) Hg-fraction containing nucleotide other than ATP and ADP, and (iv) alcohol fraction inclduing glycerophosphate, glucose-1-phosphate and glucose-6-phosphate, from Ba-soluble fraction. The following results have been obtained: (1) Remarkable differences are not observed in the distribution of P^{32} of all the fractions among normal, pair-fed and thiamine-deficient rats; (2) the specific activity of labile P of ATP and ADP is about 80% of that of inorganic 1 hour after the intraperitoneal injection of P^{32}. (This value is much higher than that reported by Kaplan and Greenbergs and shows almost the same value as that of Sacks); (3) the liver of thiamine-deficient rats shows a significant decreases in turnover rate of ATP, ADP fraction, expressed in relative specific activity of both Hevesey and Sacks, compared with the values of normal and pair-fed control rats, and this decrease is restored to normal range in the recovered rats. No remarkable change in the renewal rate is observed in other acid-soluble fractions, but some decrease is shown in alcohol fraction of thiamine-deficient and restored rats as compared with normal and pair-fed control rats.

Yoshiaki Miura: How many counts per minute (c.p.m.) were obtained in respective fractions, when P^{32} of 15 mC was administered to a rat? Is it possible to remove off a triace of P^{32}, by Lehninter's method, especially when highly radiaactive P^{32} is used?

Kikuo Ogata: The c.p.m. (mean value of 5–10 minutes) of Hg-fraction, which was the smallest of all acid-soluble fractions, was about 100, and the c.p.m. of background was about 20. So we think 15mC per 100 g. body weight was suficient to measure the renewal rate of all acid-soluble fractions of the rat liver. The method of Lehninger was proved to be satisfactory to precipitate the contaminated inorganic P from the phosphate fractions by the preliminary experiment, in which the inorganic $P^{32}O_4$ was added directly to the liver homogenate which was not injected with P^{32}. As other organic fractions, the ATP, ADP fraction was almost free from the contamination of inorganic P. A trace of the contamination was proved to be removed satisfactorily by our modification of Lehninger's method.

Haruhisa Yoshikawa: "Relative specific activity" of Hevesy is originally calculated according to the assumption that P usually enters into cells as inorganic P, and forms "metabolic pool," which is readily available for synthetic processes. His assumption is, however, obvious. I hope you will be also careful to this obvious assumption in further investigations.

Kikuo Ogata: We employed the calculation methods of both Hevesey and Sacks to compare the renewal rate of P in acid-soluble phosphate compounds, and no remarkable difference was observed between the results obtained by both methods. We have had not yet the chance to examine the characters of Hevesey's method. But in our experiment the specifie activity of P^{32} of inorganic P showed the highest value one hour after the intraperitoneal injection of P^{32}.

120. Correlations between the energy metablism and the motility of spermatozoa. I. Observations on the anaerobic glycolysis of the boar spermatozoa. BY TAKAMITSU SEKINE, GEN'ICHI NOZUE, HIROTSUNE TAKAHASHI, MIDORI FURUKAWA AND HARUHISA YOSHIKAWA(*From the Department of Biochemistry, Faculty of Medicine, University of Tokyo, Tokyo*). The study on the dynamic correlations of the following three cycles of the spermatzoa was undertaken: glycolysis as the energysupplying cycle; phosphorus metabolism as the energy-transferring and- storage cycle; and the stimulatous-motile mechanism as their function. Anaerobic glycolysis activities were measured with CO_2 evolutions by Warburg apparatus in the medium of Ca-free Krebs bicarbonate-Ringer solution, and the lactate formed was estimated colorimetrically by the Barker-Summerson's method. The results were sumarized as follows: (1) Z_{G2}^{N}, CO_2 μl. evoluted/100 millions spermatozoa/hour, was 11.5 on the average, about one-sixthc ompared with that of bovine spermatozoa; (2) CO_2 evohition was protportional to the concentration of boar spermatozoa for first 20 minutes when the concentration was up to 700 millions/ml. and for 60 minutes when the concentration was up to 400 millions/ml; and (3) Z_{L2}^{N}, mg. lactic acid formed/100 millions spermatozoa/hour, was 0.031 on the averge. Thus CO_2 calculated from lactate/CO_2 evoluted was 0.74. This difference (26%) may be attributed to the metabolic CO_2 from some decarboxylation reactions or the formation of other acids than lactic acid. In addition, the motion of boar spermatozoa was stifled completely by $10^{-2}M$ nicotine, $1.5\times10^{-1}M$ tetraethylammoniumbromide, $10^{-2}M$ pilocarpine, $10^{-3}M$ strychnine, $10^{-2}M$ quinine, $10^{-3}M$ quinidine, $10^{-2}M$ cocaine, $10^{-2}M$ brovaline, $10^{-3}M$ baribital, $10^{-2}M$ luminal, $0.2\times10^{-3}M$ digiotonine, $10^{-2}M$ chloroform, among 30 kinds of neurotropic substances investigated.

121. Correlations between the energy metabolism and the motility of spermatozoa. II. Phosphorus metabolism of boar spermatozoa. By GEN'ICHI NOZUE, HIROTSUNE TAKAHASHI, MAKOTO NAKAO, TAKAMITSU SEKINE, MIDORI FURUKAWA AND HARUHISA YOSHIKAWA (*From the Department of Biochmistry, Faculty of Medicine, University of Tokyo, Tokyo*). Boar spermatozoa were used to show how much P^{32} could be incorporated from the medium into the spermatozoa. Spermatozoa were washed thoroughly and rapidly by using a filtering apparatus which required only a few minutes to separates permatozoa from their surrounding medium, and the content of P^{32} in the spermatozoa was estimated. In the aerobic condition, the incorporatio ncurve attained the constant level of 2% incorporation in 60 minutes or so at 37°, which lasted for 3 hours there-after. In the anaerobic condition (5% CO_2 and 95% N_2) the curve attained an almost constant level in 10 to 60 minutes and after that it gradually rose and reached the level of 0.6% of incorporation in 2 hours. The phosphorylation mechanism was demonstrated to take place more vigourously in the air than in the anaerobic condition. Next, each specfic activity of acid-soluble, lipidic, nucleic acid and protein fractions of spermatozoa was calculated from time to time from the data obtained by the estimation according to Shneider's method. Radioactive P^{32} was not detected at all in the nucleic acid and protein fractions. It suggests that neither nucleic acid nor protein is synthesized by spermatozoa in this condition. In the acid-soluble fraction, radioactive P was soon saturated in the aerobic condition, and its specfiic activity was only about 0.5% of that of the inedium. The reason, why the turnover of P is so small than expected, may be that the P of an only small part of the cell body is exchangeable with extracellular P or that only few of the acid-soluble P-compounds in the cell are exchangeable with inorganic P.

122. Studies on the regulation mechanisim of myofibril contraction induced by ATP. By KOHEI HAYASHI, GENKICHIRO TAKAGAI AND SHOSUKE OKAMOTO (*From the Department of Physiology, Keio University School of Medicine, Tokyo*). In order to clarify the physiological regulation mechanism of myofibril contraction induced by ATP, myofibrils were prepared from rabbit or toad muscles by universal homogenization, and washing with 0.05 *M* K-phosphate buffer at 0°. The myofibril contraction was microscopally observed. In 0.25 *M* KCl or below, myofibrils contracted, but not in the presence of K-salt of ATP. A trace amount of Mg was found to be required for the myofibril contraction by ATP; but 10^{-3} *M* $CaCl_2$ rather inhibited the contraction. In the presence of 10^{-3}*M* $MgCl_2$, Ca gave no inhibition. The following oxidizing agents showed the inhibition of the myofibril contraction by ATP: 10^{-6}*M* $KMnO_4$; 10^{-5} *M* benzoquinone; 10^{-4} *M* quinhydrone; 10^{-4} *M* β-methylnaphthoquinone; 10^{-3} *M* methylene blue; 10^{-2} *M* 2,6-dichlorophenol indophenol; 10^{-3} *M* H_2O_2: 19^{-2} *M* KI and 10^{-2} *M* oxidized ascorbic acid. But this is not the case with 10^{-2} *M* $K_3Fe(CN)_6$ and 10^{-2} *M* $K_2Cr_2O_7$. It is of interest that, after removal of this substance by centrifugation the in-hibition by oxidized ascorbic acid, can be reversibly eliminated by 10^{-2} *M* reduced ascorbic acid or $10^{-2} M$ cysteine. These results will suggest that such an oxidation-reduction system may be concerned in the regulation of the muscle contraction *in vivo*.

Junkichiro Hirade: For the reason why the contraction of actomyosin-threads is inhibited by oxidizing agents and reactivated by reducing ones, it certainly concerns the state of oxido-reduction of -SH groups, and furthermore, the role of -SH groups might be considered as if to give the site of combination for ATP. To acertain the above stated hypothesis, it is hoped to assay the -SH groups at each stage of reaction.

123. Study on the free phosphate formation in a frog sartorius. By KAZUO NISHIO (*From the Department of Hygiene, Osaka University, Medical School, Osaka*). In order to decide whether the formation of the free phosphate may be due to only the breakdown of phosphocreatine, the loss of creatine and the loss or uptake of inorganic P were determined as follows: Two pieces of frog sartorius were immersed in 20 ml. of Ringer solution, contianing 4 μg. P/ml. for about one hour after the isolation from a frog, and then in a new Ringer solution for next one hour; thus the same procedure was repeated. The immersed muscle was allowed to work in air or oxygen by direct electric stimulus in such a way as to contract by continuous twitches at intervals of 0.5 seconds or as to repeat 2 seconds tetanus after 3 seconds rest, untill the contractility in the period between third and fourth immersion disappeared. Under anaerobic condition the uptake of inorganic P by sartorius appeared only in other seasons than summer and only in a certain period after isolation. The loss of inorganic P by resting sartorius had no parallel relation to the loss of creatine, but the former was larger than the latter in molecular ratio except the period of phosphate uptake. The work by twich showed no remarkable increase in the losses of both inorganic P and creatine by sartorius; the work by tetanus increased several times the losses of both inorganic P and creatine as much as that by resting sartorius as the control. Under aerobic condition the work by tetanus resulted in far more increases in the loss of inorganic P than that under anaerobic condition; while the increase in creatine loss could hardly be approved as compared with the resting sartorius. These facts indicate that the uptake of inorganic P by isolated frog muscle takes place unless active metabolism is exhibited at least in a solution of low P concentration, and that this behavior of phosphate penetration into the muscle *in vitro*, either into intracellular or into extracellular space, is remarkably different from that *in vivo*, which has been established by isotopic studies. It can be suggested also by this experiment that the formation of inorganic P is so complicated that the breakdown of phosphocreatine should not be a sole reason of the free phosphate liberation both in rest and in contraction, inspite of a difficult problem of permeability of muscle cells.

Haruhisa Yoshikawa: Such a relation between the losses of creatine and phosphate can be supposed naturally from the dynamic process in phosphorylation of many phosphor-ester in muscle.

Kazuo Nishio: This experiment had the idea that such a supposition must be researched actually in relation to the physiological function of muscle.

Genkichiro Takagaki: The permeability

of muscle cell must be taken as an important nature to produce your experimental facts.

Kazuo Nishio: Of course I took care of the permeability, but the losses of creatine and free phosphate from muscle showed irregular increase or decrese in relation to time elapsing after muscle isolation, so the permeability can be sole nature to produce my results.

124. Biochemical and histochemical studies on nervous diseases. I. By SHIGEO OKINAKA, MASAKI YOSHIKAWA, TOSHIJI MOZAI, MASATERU TOYOTA, FUJIO NAKATA, JUYA GOTO, SHIGERU NAGAYAMA, KAZUO SHIZUME, HIROSHI IBAYASHI, AKIRA OGAWA, NOBUTERU YAMADA and HIROMICHI NISHIMIYA (*From the Medical Clinic, School of Medicine, University of Tokyo, Tokyo*). *I. Cholinesterase (ChE)*: In 5 cases of nephrosis the serum ChE activities always increased. The clinical observations of Willson's disease (hepatolenticular degeneration) suggest a close correlation between liver and basal ganglia of brain stem. The present authors have demonstrated the decreased serum ChE activity in liver diseases as well as an extraordinarily high content of ChE in basal ganglia. There was, however, observed no remarkable difference in the ChE activity of basal ganglia between liver diseases (14 cases) and the control (14 cases). In 2 cases of myasthenia gravis diisopropylfluorophosphate (DEP) gave stronger inhibitory effect on ChE than prostigmine did. With the blood speciments of myasthenia gravis (2 cases) and muscle atrophy (2 cases), parallel relationship was observed between clinical symptoms and the blood ChE activity, especially the erythrocyte ChE activity. *II. Choline Acetylase*: With different parts of fresh dog brain both choline acetylase and ChE activities were determined. The contents of both enzymes were greater in the order of *Ncl. caudatus*, cerebellar cortex, cerebral cortex; but the difference in ChE was more marked than choline acetylase. *III. Copper Metabolism in Willson's Disease*: With a male patient (11 years) of Willson's disease, there was a remarkable increase in urinary excreion of copper which could be augmented by the injection of BAL. In Willson's disease, the copper content of liver was generally 3—5 mg./100 g. dry weight and seldom exceeded 10 mg. %. Histochemical examinations of the copper distribution in 36 pathologic livers revealed copper granules in parenchymal cells in only 3 cases: Willson's disease, Laennec cirrhosis and subacute liver atrophy. In the case of Willson's disease, some liver lobules showed a large amount of diffusely stained copper granules. These granules will indicate the abnormal copper metabolism.

Symposia on Plasma Proteins

1. Studies on the physico-chemical and functional properties of blood plasma proteins. By KEIZO KODAMA, HIDEMATSU HIRAI and 17 other members. (*From the Department of Biochemistry, Faculty of Medicine, Tokyo University, Tokyo*) *I. Problems on the Structure of Plasma Proteins*: (1) When serum albumin and γ-globulin were hydrolyzed with 0.5 N NaOH at 100°, the colortion of the hydrolyzates by phenol reagent attained its maximum about 5 minutes' hydrolysis. The increase in colortion mentioned above was mainly due to the liberation of phenolic hydroxyl groups of the proteins during hydrolytic process. Phenyl acetate, acetyl tyrosine and benzoyl tyrosine were hydrolyzed under the similar conditions, and it was found that after about 5 minutes' hydrolysis the hydrolyzate reacted with phenol reagent to give colorization which could be interpretable on the basis of complete liberation of phenolic hydroxyl groups. These facts show that some phenolic hydroxyl groups of the proteins are in free state and others are in bound state in the protein molecule. Moreover, because of the fact that free carboxyl groups of the proteins were markedly increased by denaturation with dilute alkali, the ester linkage between phenolic hydroxyl and carboxyl groups in the protein molecule might be assumed. (2) Coloration with Sakaguchi's reagent of the hydrolyzate of serum albumin and γ-globulin by mineral acid increased with the time of hydrolysis. It can, therefore, be deduced that some of the guanidino amino groups in the proteins are free and others are bound with carboxyl groups by peptide linkage. (3) The amounts of iodine consumed by serum globulin were increased by alkali, heat and heat-alkai denaturations of the protein. While, in the case of serum albumin, the amounts of iodine consumed incresed only in the case of heat-alkali denaturation. Activation energy of iodination of serum globulin was larger than that of serum albumin, and that of the denatured protein was smaller than that of the native one. *II. Hydrolysis of Plasma Proteins*: (1) When serum albumin and γ-globulin were hydrolyzed by 1 N H_2SO_4 at 100°, amino N increments in percentage were about the same order of magnitude for both proteins, but percentage increase in N.P.N. was larger in the latter than in the former protein. (2) Among serum proteins, serum albumin was found to be most easily hydrolyzable by various proteolytic enzymes, fibrinogen came next and β-and γ-globulins were most resistent to enzymatic hydrolysis. The rate of enzymatic hydrolysis did not change appreciably by heat denaturation in case of serum albumin, while that of serum globulin increased by the same treatment. *III. Binding of Dye Ions by Serum Proteins*: The maximum number of methyl orange bound by serum albumin was found to be about 8 per molecule of the protein. Changes in the amount of bound dye with pH and formol treatment indicated that basic groups in albumin molecule might cause the binding. The amount of bound dye was decreased by heat treatment of the protein and was still detectable even after treatment at 50°. A/G ratio of serum could be easily calculated from the amount of bound dye and the total protein concentration, since serum globulins did not bind dye ions. *IV. Production of Hemoglobin in the Culture of Bone Marrows and the Effects of the Addition of Serum Proteins and Amino Acids*: Production of hemoglobin in the culture of rabbit bone marrow was enhanced by the addition of human serum globulin and the yield was diminished by the addition of human serum albumin or by the elimination of hisdidine, tryptophan, and phenylalanine from the culture medium. *V. Purification and Clinical Applications of Antibody Globulins*: Fraction containing antibodies were determined by salting-out with $(NH_4)_2SO_4$ and Na_2SO_4, and by electrophoresis, on normal human serum and anti-diphtheria, whooping cough, and *Encephalitis japonica* sera of human being, goat, horse, rabbit, *etc.* Antibodies were purified by salting-out with Na_2SO_4 on the basis of the above mentioned determination and they were proved to be clinically applicable to measles, whooping cough, *Encephalitis japonica*, and diphtheria. γ_2-Globulin prepared from anti-whooping cough horse serum gave marked therapeutic effects on whooping cough.

2. **Studies on plasma protein based upon the Takata reaction and flocculation reaction.** By MAKI TAKATA AND SHIZUO TAKATA (*From the Laboratory of Medical Chemistry, Toho University Medical School, Tokyo and the 2nd Medical Clinic, Kobe Medical College, Kobe*).

3. **Physiological changes of serum and its fractions.** By HISAHITO YOSHIMURA AND BUNYA EGUCHI (*From Department of Physiology, Kyoto Prefectureal College, Kyoto*). (1). In summer the circulating volume of blood increases and the concentration of serum protein decreases. This decrease is due to the dilution of blood vessels in hot climate, resulting in a dilution of serum by protein tissue fluid. (2). The A/G ratio of serum decreases in summer and increases in winter. The concentration of γ-globulin also decreases in hot climate. This fact is probably related to the decrease in host resistance against infection. (3). The seasonal changes of serum protein and of its fractions are reduced in the subjects of old ages. (4). The lower is the protein content of habitual diets, the lower is the concentration of serum protein. Such a decrease in serum protein is due to those of albumin and γ-globulin. (5). The sterilizing ability of serum against *Trypanosoma* is proved to be closely related to its γ-globulin content.

4. **Factors affectung the distribution of serumprotein fractions.** By HIDEO MABUCHI (*From the Department of Biochemistry, Kobe Medical College, Kobe*). A number of experiments performed during past several years bore the following results on the role of the autonomous nervous systems and liver on this problem: (1). The subctaneous injection of pilocarpine (1 p.c. soln., 1 ml. per kg.) or ergotamine (1 p.c. soln., 1 ml. per kg.) to the rabbit increases the total serum protein and albumin fraction, but rather decreases globuins; (2). Either atropine (1 p.c. soln. 0.1 ml. per kg.) or adrenaline(1 p.c. soln. 0.1 ml. per kg.) acts exactly opposite way to the cases in (1), *viz.*decreases the total serum-protein and albumin fraction, but increases globulins; (3). The injection of pilocarpine or atropine to the dogs which kept alive by intermittent injection of glucose after the total excision of liver gave the similar effects observed in the two previous instances. These results clearly indicate the existence of antagonism between the sympathetic and parasympathetic nervous systems with regard to the serum protein and the two fractions, in the sense that any superiority in the tonic state of one of the autonimic nervous systems causes the remarkably definite deviations on both the serumprotein and the two fractions from their normal levels; and moreover show that those alterations could be provoked extrahepatically. Another series of observations concening muscular exerice or fatigue, and high grade anoxaemia revealed the similar distribution tendencies to the application of pilocarpine to the three elements—thus providing an inviting suggestion that oxygen want in tissues due either to exercise or simple anoxaemia may provoke a superiority of parasymathetic tonus.

Torao Kawachi: We have obtained the following results concerning the correlation between exercise and the contents of serum protein and hemoglobin before and after the exercise. Serum albumin content increases, but serum globulin content either remains unchanged or decreases to some extent after exercise at room temperature for the energy respiratory quotient of 5.4 or less. But for the energy respiratory quotient of 9.5, it is increased in serum globulin and albumin content.

5. **Plasma protein and hemoglobin as criteria for protein deficiency** By JUGORO TAKEUCHI (*From the Department of Internal Medicine, University of Tokyo*). Of plasma protein and hemoglobin, which will reflect the deficiency of protein more sensitively? This is a question of great interest not only from theoretical but also from clinical points of view. The author has reached a conclusion which is contradictory in some respects to the Hirade's opinion that hemoglobin is a highly sensitive index of the protein deficiency as compared with plasma protein. From the laboratory findings on under-fed human subjects (all male; 22 cases; average daily 1,600–1,700 kcal.; daily intake of protein, approx. 50–60 g.) and the experimental results on protein deficiency (5 dogs and 4 human subjects), the author advanced the following view: (1). It is verified that the concentration of the total plasma protein does not reflect the deficiency of protein so sensitively as the concentration of hemoglobin or albumin; (2). However it is hardly accepted that hemoglobin is, as compared with albumim, always a better index for the protein deficiency. This seems to hold true not only with its concentration but also with its total circulating amount. No substantial difference is found in the result between human subjects and dogs. No specificity of species as emphasized by Dr. Hiraide is observed; (3). In a few cases of protein deficiency, contratry to his observation, the author failed to note such a phenomenon, but it has been found that the decrease in the concentration of hemoglobin is always accompanied by the decrease in the total amount of circulating hemoglobin; and (4). In conclusion, it is maintained that the simultaneous measurement of both albumin and hemoglobin, seems to serve for the purpose to the best, if the criteria of protein deficiency is to be looked for in blood. That the simultaneous determination of the total amount in circulation as well as of the concentration is more desirable than the measurement of the concentration alone will no need further reiteration.

Junkichiro Hiraide: I am ready to accept the results of your experiments as a whole. However, I think, the significance of hemoglobin which I emphasized is also obvious.

Hisahito Yoshimura: It is admitted that no pseudoanemia will be observed in the experiments conducted under such conditions as authors have described. However, authors may well be reminded that pseudoanemia will be noted in cases of D. Hiraide's experiments in which the deficiency of protein is less advanced than in the cases handled by the authors.

Kaichiro Kuroda: In the case of hypoproteinosis, hydremia due to the abnormal water metabolism can be recognized together with hypoalbuminemia and anemia. As a total result of all these factors, it occurs the decrease of solid components of blood, namely the increase of blood water. Therefore I have been using the blood water content as the criterion of judging the state of malnutrition.

Junkichiro Hirade: My opinion hitherto is based on the evidence that the hemoglobin level is, as a criterion for protein deficiency or malnutrition, essentially more sensitive than the total plasma protein. Therefore, I have nothing to oppose against your own findings concerning the comparison between Hb and plasms albumin. The simultaneous measurements of both items are recommended essentially as the most suitable criterion for protein deficiency. But, notwithstanding your findings, the nutritional significance of Hb reflecting the integrated dietary conditions, stands out of question.

6. Several physico-chemical facts about plasms. By YOSHITAKA KATSU (*From the Department of Bio-physicochemistry, Kyoto Prefectural Medical College, Kyoto*). Colloid osmotic pressure of plasma is greater than that of serum Of the reduction power a similar relation has been recognized. When sol changes to coagel the concentration of solute in the dispersion medium increases. From the fact that plasma is thicker than serum, the colloid osmotic pressure of fibrinogen in plasms can be determined. When ether is added to the suspension of charcoal powder and well shaken, charcoal is transmitted to ether layer; while when char coal powder pretreated with the plasma diluted above " a certain concentration," charcoal is not transmitted. The amount of H_2O_2 decomposed by blood at " a certain concentration " is smaller than that of spontaneous decomposition of H_2O_2. Boundary potential difference between air and plasma changes at " a certain concentration " of plasma, giving a inflection point. The above-mentioned " a certain concentration " corresponds in all cases to about 1000 times dilution of plasma, which seems to be a specificity point of plasma. Red blood corpuscles suspended in a cane sugar solution agglutinate by the addition of crystal-violet. The critical concentration of agglutination decreases in case of shock, but rather increases in case of anaemia. It is probably due to an opposite changes of plama-protein in both case.

7. On the gelification of serumes, especially on the formol- and lacto-gelification. By SHO MIYAMOTO, KAORU NISHI, JIRO TANDA and YASUO CHIBA (*From the Biochemical Laboratory, Tokyo Medical-Dental Uiversity, Tokyo*). The process of formol-gelification of the serum of Kala-azar has been studied by Miyamoto and Suzuki, and that of the serum of tuberculosis by Miyamoto and Higuma. According to these observations, the augmentation of viscosits of serum by the in fluence of formalin occurs most profoundly in an extremely narrow limit of pH, namely around 6 to 8. Furthermore, they have found that this augmentation of viscosity is closely connected with "Napier-reac tion." The attitude towords formolgelification taken by each serum protein fraction was investigated by following the changes of viscosity in wide pH range till the gelification took place. It has been observed that in so far as one drop of formalin is added to 1 ml. of protein solution, only englobulin is subjected to the influence of formalin; in this case a pronounced increase of viscosity occurs in very narrow pH range between 6 and 8. On applying a larger quantity of action of formalin, in which case, a particular increase of viscosity or gel-formation is recognizable in narrow pH limit around 4.8. The viscosity curve of euglobulin changes, in the proportion to the augmentation of formalin quantity, as follows: At first the peak of the viscosity curve around pH 6 to 8 lowers and the viscosity curve ascends also in the narrow limit around pH 5.2; still at higher formalin concentrations, the peak of the viscosity curve around pH 6 to 8 disappears and the increase of viscosity together with of coagulation are recognizable only around PH 5.2. Tanda has applied this method to clarify the alcal idenaturation of eugolobulin and he has observed that the changes of the viscosity curve, which are quite similar to those in the case of the augmentation of formalin quantity, are recognizable when protein solution is treated, beforehand, above pH 10. The electrophoretical observation of serum has revealed that by the effect of formalin, the part corresponding to γ-globulin decreases and the part corresponding to β-globulin rather increases. The Miyamoto-Suzuki's method used in place of formalin to observe the viscosity change in 40% diluted lactic acid added serum of pleursy patients over a wide range of pH value. In 40% diluted serum a characteristic rise in viscosity was observed around pH 4.8–5.0, while this was not observed in the serum of healthy persons. This rise in viscosity was confirmed by electrophoresis, exhibiting a close correlation to γ-globulin. Furthermore, each fraction of the horse blood serum protein and their gelformation were studied: Lactic acid had no morked effect on albumin and pseudoglobulins I and II; while euglobulin containing γ-globulin revealed coagulation and precipitation at pH 4.6–6.0 and euglobulin added-albumin revealed gelformation instead of coagulation.

8. On the combination between serum albumin and congo red. By KENZO TORII (*From the Deqartment of Biochemistry, Nara Medical College, Nara*).

9. Experimental studies on plosma protein. By RYOJI ITOH (*From the Department of Medical Chemistry, Nihon University, Tokyo*). (1). Along with the antibody production, albumin is sometimes diminished, which makes us hardly believe that the albumin may take any part in antibody production. (2). In general contrary to the conspicious increase of γ-globulin, there is a marked decrease of α-globulin, which makes us surmise that α-globulin directly participates in generation of the former. (3). β-Globulin reveals no pronounced change of the generation of antibodies. However, as to such special proteins as the O-antigen of *S. typhi* 0-901-W strain, the increase in β-globulin is noticed in the time of antibody production. This fraction may constitute a part of the antibody (4). General erally, a parallelism can be seen between antibody titers produced by the injection of antigen and the amounts of γ-globulin. Besides the specfic γ-globulin, non specific γ-globulin fractions rise and fall in parallel with the increase and decrease of the specific ones. Namely, in γ-globulin fractions increased at the time of antibody generation, the enhanced non-specific fractions without relation to the antibody are contained. (5). Reticuloendothelial and lymphaticsystems participate in the generation of γ-globulin. (6). No relationship has been observed between γ-globlin and protective enzyme. (7). The increase in γ-globulin fractions is caused by the stresses such as antigen injection, incision or partial resection of liver, liver disturbance induced by administration of CCl_4 and ligation of pancreatic ducts.

10. Immunochemical studies on diphtheria antitoxin. IV. The effect of iodination on the split antitoxin. By TADAO KATSURA (*From the Institute for Infectious Diseases, University of Tokyo, Tokyo*). Equine diphtheria antitoxin was highly purified by the peptic digestion of toxin-antitoxin floccules, followed by the ammonium sulfate fractionation. Thus purified antitoxin had about 800 Lf units per mg. protein N, and it was proved to be electrophoretically homogeneous, the stability range of which was found between pH 4.0 and 9.6. The effect of iodination on the antitoxic activity was studied to elucidate the nature of the immunologically activity structure, using the purified split antitoxin. The antitoxin solution was iodinated at pH 9.2 9.4 by the addition of $N/10$ iodine solution. After the addition of an excess $Na_2S_2O_3$ the reaction mixture was dialyzed for 40 hours. The antitoxic activity and protein N of the iodinated antitoxin was estimated. Iodine was estimated by the micro-modification of Leipert's method according to Haurowitz. Colorimetic determination of tyrosine was also carried out by the micro-modification of the Millon-Weiss method. Along with the iodination process, the antitoxin was inactivated without apparent denaturation. About 85 % or more of antitoxin activity was destroyed when the reaction proceeded to an extent of about 30 iodine atoms per protein molecule (the assumed molecular weight, 11,3000). The doubt remained whether the inactivation was due to the direct alteration in the specific part of the antibody rather than elsewhere in the molecule or due to the indirect, non-specific changes of physico-chemical properties of the antitoxin. The analysis of the toxin-antitoxin floccule, however, showed that the partially altered antitoxin solution gave almost the same amount of specifically precipitable antibody N per Lf unit as that of the untreated one, and that the iodine amount per mg. antibody N derived from the floccule was found to be approximately equal to that of the altered antitoxin. Thus the presence of the iodinated antitoxin retaining the combining power with toxin was suggested. The dissociation of the iodinated toxin-antitoxin complex by peptic digestion followed by the ammonium sulfate fractionation gave the iodinated antitoxin (to the extent of about 50 iodine atoms per molecule) without much loss of its activity (600 to 700 units per mg. protein N). From these results, the inactivation of antitoxin by iodination seems to be due to the direct alteration in the specific part of the antitoxin molecule. Thus, it may be concluded that there is a group, probably tyrosine residue, within the specific combining site of the antitoxin which is altered by iodination.

11. Despeciation of horse serum globulin by Takadiastase. By TSUNEHISA AMANO and SHINZO ISOJIMA (*Form the Department of Bacteriology, Medical School of Osaka University, Osaka*). (1). Globulin fraction of antidiphtheric horse serum was digested by Takadiastase at 37° for 96 hours. The yield of this final product was 38.2%, on the calculation basis of the antitoxin unit by Ramon's flocculation test and Eyrlich's method. The despeciation factor of this final product was at least gerater than 100. This final product was electrophoretically one component. There was little difference between this final product and normal horse serum globulin in respect to the antigenity with ring test of the precipitin reaction. The final product hardly developed the flocculent precipitates or turbidity with antihorse-serum rabbit serum. (2). Normal horse serum was digested with Takadiastase and dialyzed against distilled water for one week, but this concentrated dialyzate did not act as a hapten. The digested serum was deproteinized with ethanol or acetone, but this filtrate also did not behave as hapten for the sensitized guinea pigs. (3). The acid-phosphatase contained in Takadiastase was completely inhibited by 0.2 *M* NaF, or adsorbed on caolin. In these cases despeciation was observed, but this is not the case with a component obtained from *Aspergillus oryzae* containing much acid-phosphatase but little protease. (4). The crystalline Takaamylase showed no despeciation of the antidiphtheric horse serum. (5). It is believed that two kinds of protease exist in Takadiastase: the one is acid-protease and the other is alkaline-protease. With the digested antidiphtheric horse serum by Takadiastase at pH 7.5, despeciation of serum did not occur. The filtrate obtained from Takadiastase by the addition of acid clay, and by shaking for 1 hour at pH 3.5, successively, contained acid-protease, but not alkaline protease: this filtrate was still active for horse serum but not for gelatin and casein. From the results obtained it is suggested that the acid-protease may be responsible for the despeciation of the serum.

12. The rabbit antibodies by electro phoretic and quantiative precipitin methods, and the fractionation of globulin. By MASANA-OGATA AND YOSHIO MOCHIZUKI (*From the Department of Hygiene, Okayama-University School of Medicine, Okayama*). (1). Using simple and mixed antigen systems, the serum of hyperimmune rabbit showed an increase in the total protein and γ-globulin concentration in comparison with the normal rabbit serum. Employing Heiderberger's method, quantitative precipicin reactions and quantitative complement fixation were examined with yeast polysaccharide, crystalline egg albumin and bovine thyroglobulin systems as follows: Yeast polysaccharide, $\mathrm{AbN}=10.6\,\mathrm{Yp}-26.8(\mathrm{Yp})^2$ and anhydrous substance, $\mathrm{AbN}=11.7\,\mathrm{Yp}-32.7(\mathrm{Yp})^2$; crystalline egg albumin, $\mathrm{AbN}=18\,\mathrm{EaN}-135(\mathrm{EaN})^2$; and thyroglobulin, $\mathrm{AbN}=5.6\,\mathrm{TgN}-25(\mathrm{TgN})^2$. The antigen/antibody ratio in equivalent zone obtained by the succesive injections of a small quantity of antigen was higher than that obtained by the normal immue method. The antibody purified by the special method was electrophoretically proved to consist of γ-globulin. The highly purified antibody was obtained by electrophoretical γ-globulin separation method and convective electrophoretic method. The electrophoretical results with the supernatant of antigen-antibody reaction showed that the globulin of immune rabbit serum gave partial precipitation following to antigen-antibody reactions. By examinaiton of antigen-antibody reactions with the supernatant a remarkable decrease in the titer of antidody was ascertained. The partial precipitation (17–35%) of γ-globulin of immune rabbit serum could be demonstrated by the fact thatγ-globulin concentration estimated by electrophoresis was greater than protein concentration and was precipitated by quantitative precipitin method. The supernatant from the equivalent zone contained a relatively larger quantity of

γ-globulin. (83–65% of antibody γ-globulin). It was to be inferred therefore that γ-globulin of immund rabbit serum contained antibody feeble in reactive power (2). The electrophoretically simple γ-pseudogloblin was isolated by means of the rotating membrane method for salting out of γ-globulin, followed by fractionation with 1.39, 1.86 and 1.22 *M* ammonium sulfate, successively (three times). γ-Globulin of 90–95% purity was isolated in the process of twice repeated purification of the original serum by convective electrophoresis. Thus isolated γ-globulin and albumin showed immunological specificity as antigen. By convective electrophoresis, the samples isolated by the Cohn's fractionation (II+III) could be further purified.

13. **Polarographic study of serum protein.** By SAKIO SASAI and MASAO EGAWA (*From the Medical Clinic, Kyoto University, Kyoto*).

Symposia on Nucleic Acid and Nucleoprotein

1. **Studies on spleen nucleoprotein. III. On the isolation of nucleic acid and nucleoprotein of pentose-nucleoproteide.** By AKIRA INOUE and SHIZUO OKUYAMA (*From the Research Laboratory for Protein, Yamaguchi Medical College, Ube*).

2. **Studies on spleen nucleoprotein. IV. On desoxynucleoprotein.** By AKIRA INOUE and SHIZUO OKUYAMA (*From the Research Laboratory for Protein, Yamaguchi Medical College, Ube*).

3. **Nucleic acids and related compounds of Tiai (fish red muscle).** By KIYOHIKO UMEMURA and NOBUO SUZUKI (*From the Department of Biochemistry, Nagoya University School of Medicine, Nagoya*). *Tiai* has been confirmed by the author to resemble the heart muscle in respects to enzyme systems (cyclophorase, cytochrome and enzymes for amino acids) and the ATP ase activity. With *Tiai*, ordinary muscle, liver and heart, the contents of P was determined after fractionation as follows: *Acid Soluble P*—ordinary muscle 150, other tissues 100 mg. %; *Lipidic P*—*Tiai* 75, heart and liver 65, ordinary muscle 30 mg. %. (In case of liver, lipidic P varies from 60 to 80 mg. %. in parallel with its color intensity); *RNA P*—liver 65, heart and *Tiai* 25; ordinary muscle, 8 mg. %; *DNA P*—liver and heart 15–20, *Tiai* 10, ordinary muscle 2 mg. % g. On the other hand, the existence of numerous mitochondria was observed in *Tiai* independently, and in this respect the data of respiratory enzymes and lipidic P are closely related. It is remarkable that *Tiai* contains RNA and DNA as much as heart, and far more than the ordinary muscle, indicating that *Tiai*-may have some synthetic activity for the purpose of persistent operations. After the injection of $MgSO_4$ the acid soluble fraction of respective tissues was analyzed for P contents, where CCl_3COOH was added prior to homogenization to prevent the destruction of ATP: (a). *Ba-insoluble fraction*: *Ionrganic P*—Ordinary muscle 80; *Tiai* 45, liver 30, heart 20 mg. %; *ATP plus ADP*—Ordinary muscle 40, *Tiai*, heart and liver 20 mg. %; *HD P*—heart 15, *Tiai* 8, liver and ordinary muscle 3 mg. %. (b). *Ba-soluble alcohol-insoluble fraction*:

Creatine P—ordinary muscle 12, *Tiai* 8, heart and liver 4mg.%; *Glucose-1-phosphate P*—all 3 mg.%; *Fructose-6-phosphate P*—all 2-3 mg.%; *AMP P*—all 5mg.%. (c). *Ba-soluble alcoholsoluble fraction*: Heart 15, *Tiai*, 12, ordinary muscle and liver 5 mg. %. As a whole, *Tiai* has been shown to possess an intermediary character between heart and ordinary muscle, especially in regard to the contents of inorganic P, HD P and creatine P. Of the amino acids estimated, glycine was abundant in both *Tiai* and ordinary muscle. From the results obtained, the basic structure of these two muscles are considered to be analogous, which suggest that they may come out of the same origin. Thus it can be assumed that *Tiai* may be transformed by adapting itself to its activity.

4. Combination of ATP and sulfate. By TOKURO SATO, TAEKO SUZUKI AND HARUHISA YOSHIKAWA (*From the Division of Nutrition and Biochemistry, the National Institute of Public Health, and the Department of Biochemistry, Tokyo University, Tokyo*). A peculiar reaction of sulfate with ATP was recognized incidentally in the course of study on phenyl sulfate formation in rat liver. (1). When 5 ml. of an aqueous solution containing 2 mg. of ATP and varying amount of sulfate were mixed with $BaCl_2$ solution, the sulfate failedp to recipitate if the amount of sulfate was less than equimolar to ATP. However, when HCl was added to the above clear solution, $BaSO_4$ began to precipitated. This phenomenon indicates that the combination of ATP and sulfate is acid labile. ATP could be used as Ca or Na salt without any difference in the result. The same result was also obtained by using bezidine as the precipitant of free sulfate at pH 3. If ATP was hydrolyzed in dilute alkali (0.03 N NaOH), such a combining capacity disappeared within 1.3 hours. (2). Flasks containing 5 ml. of 10% liver brei in isotonic KCl solution, 0.2 M citrate and sulfate equivalent to 100 γ S were shaken at 38° in air, and then protein was precipitated with 1 ml. of 20% CCl_3COOH. After adjusting the supernatant to pH 3 free sulfate was determined by the nephero-metric method of Denis and Reed. Reduction of free-sulfate was often observed shortly after the commencement of shaking. When 0.2 ml. of 0.1 N NaF were added prior to the addition of citrate, the reduction of free-fulfate was more marked and continued for one hour. The lost free-sulfate was recovered by adding HCl. The same phenomena were seen with liver brei treated with acetone and supplemented with pyrophosphate. But in this case potassium and phosphate were indispensable. The reduction of free-sulfate seems to be due to the combination of sulfate with some substance which is very likely to be ATP.

Katashi Makino: By Boesekens's reaction you will be able to know whether sulfate combines with the hydroxy group of ribose or not. I would recommned to use radioactive S and paper chromatography for the study of phenyl sulfate formation.

Tokuro Sato: We are preparing to use radioactive S.

5. A study on adenosine-sulfates. By IKUO YAMASHINA (*From the Chemical Institute, Faculty of Science, Nagoya University, Nagaya*). It might be expected that adenosine-sulfate, having sulfate in place of phosphate in adenylic acid, would inhibit competitively not only the enzymes requiring adenylic acid but the compounds containing adenylic acid as coenzyme

(DPN, TPN, ATP, *etc.*) or could replace adenylic acid as coenzyme. Adenosine-sulfates were synthesized as follows: Adenosine dissolved in pyridine is sulfated by chlorosulfonic acid diluted with chloroform at 0—10°; the pyridine salts of adenosine sulfates are converted to the Ca-salts and precipitated by ethanol. With varying amounts of chlorosulfonic acid, mono- and di-sulfates can be obtained. Monosulfate consumes 0.7 moles periodate per mole, while disulfate none. Since the sulfur contents of these two compounds coincide their their theoretical values, respectively, it is concluded that the composition of monosulfate is " adenosine-5'-sulfate " and " adenosine-2'- or/and -3'-sulfate "; while that of disulfate is " adenosine-2',5'- or/and -3',5'-disulfate." Ethyl alcohol and formic acid dehydrogenases requiring DPN, were inhibited by adenosine-monosulfate, but not by adenosine-disulfate. Both sulfate compounds gave no effect on the O_2-uptake of baker's yeast and *Prot. vulgaris* with glucose, ethanol, or L-leucine as a substarte. Adenosine monosulfate was slightly hydrolyzed by sulfatase prepared by the autolysis of the liver of *Charonia lampas*.

6. Cleavage of nucleic acid my catalysis of lantanium hydroxide. By MICHIO SHIMOMURA (*From the Department of Chemlstry, Faculty of Science, Nagoya University, Nagoya*).

7. Studies on protamine. (On the hydrolyzing velocity of peptides and diketopiperazines. By RYOZO HIROHATA, NOBUO IZUMIYA, MITSUYUKI NAKAMURA, ATSUO NAGAMATSU AND TAKESHI ONO (*From the Department of Medical Chemistry, Kyushu University School of Medicine, Fukuoka*). In order to elucidate the cause of the occurrence of valine anhydride in the hydrolyzates of mugiline boiled with 25% H_2SO_4 for 20-25 hours, the velocity of hydrolysis of the following peptides and diketopeperazines with HCl or H_2SO_2 at various concentrations examined: at 100° has been (a). 1 *N* HCl—Glycylglycine, glycyl-DL-alanine, glycyl-DL-serine, glycyl-DL-*nor*-leucine, glycyl-L-leucine, L-leucyl-glycine, glycyltheronine, glycylallothreonine, glycyl-L-isoleucine, glycyl-DL-valine, glycyl-L-arginine, L-leucyl-L-leucine, DL-valyl-DL-valine; (b). 1 *N* H_2SO_4—DL-Alanylglycine, glycyl-DL-norleucine, L-leucylglycine, glycyl-DL-valine, DL-valyl-DL-valine; (c). 25% HCl—Glycyl-L-isoleucine, L-leucyl-L-leucine, glycyl-DL-valine, DL-valyl-DL-valine; (d). 25% H_2SO_4: L-Leucyl-L-leucine, glycyl-DL-valine, DL-valyl-DL-valine; (e). 1 *N* HCl—Glycine anhydride, glycyl-DL-alanine anhydride, glycyl-DL-norleucine anhydride, glycyl-L-leucine anhydride, glycyl-L-tyrosine anhydride, L-leucine anhydride, glycyl-DL-valine anhydride; and (f). 1 *N* H_2SO_4—DL-Valine anhydried. Findings of Levene, Abderhalden, Synge and Christensen in similar experiments were confirmed to some extent. It is noteworthy that valylvaline and valine anhydride are most resistant against hydrolysis with both HCl and H_2SO_4 among the substrates examined, and that the anhydride is not formed from glycylvaline and valylvaline even after 30 and 40 hours' boiling with 25% H_2SO_4; while unhydrolyzable leucylleucine shows no such tendency in the same hours. The above experiences were applied to hydrolyze the mugiline and fibroin for the isolation of peptides.

Addendum: (1) The velocity of hydrolysis of leucylglycine is smaller than that of glycylleucine at the beginning stage.

But in our experiments, unfortunately, the determination was not made at short intervals in the last stage, and the 100% hydrolysis of the two peptides appears to have been attained at the same time in our graph. We have to determine NH_2-N more precisely. (2) We used to make correction on NH_2-N quantity obtained by Van Slyke method if the substarte contains glycine or glycine peptide.

8. On the molecular weight of pentose-nucleic acids. BY TOMOKO KITAMURA, KOJIRO ISO AND ITARU WATANABE (*From the Institute of Science and Technology, Tokyo University, Tokyo*). The molecular weight of yeast RNA was found to be at least 20,000–40,000 by viscosimetry and by the diffusion method; and about 30,000 by osmotic pressure method. The commercial preparation of yeast RNA (Merck) gave considerably a lower molecular weight (4,400), which was probably caused by the alkali treatment during purification. In fact, RNA prepared by the Clarke-Schryver method had a molecular weight of about 30,000 at first, which was reduced to about 6,000 after the cold-alkali treatment. The diffusion constant ($D_{20,\,w}$) of nucleic acid varied greatly with the salt concentration in solvent: Merck's yeast nucleic acid, $D_{20,\,w}=14.8\times10^{-7}$ cm.^{2}/sec. in phosphate buffer (pH 7.7) at the ionic strength of 0.2; $D_{20,\,w}=40\times10^{-7}$ in water alone. Such a variation may be due to the charge effect, which can be almost completely eliminated in case of the above phosphate buffer (pH 7.7), giving the well agreeable value (14.8×10^{-2} cm.^{2}/sec.) with the molecular weight of 4,400 as rod-shaped particle. These results are quite different from those of Tsuji (1951) as tetranucleoside. The highly polymerized PNA was prepared from beef liver by the mild method of Grinnan and Mosher, and its molecular weight was calculated to be 100,000—1,000,000, thus varying greatly with the preparation methods as well as with the orgins of sample.

9. Ribonucleic acid in the fixed tissues of various animals. BY SACHIO HAMASAKI AND JIRO SATO (*From the Department of Pathology, Okayama University, Okayama*).

10. Studies on the ketoenolic substances, desoxyribonuclease and ribonuclease. BY YUKIO HAMAZAKI AND HIROMITSU TANI (*From the Department of Pathology, Okayama University Okayama*). Cold perchloric acid and trichloracetic acid can extract ketoenolic substance (KES) out of a raw tissue; but this is not the case with a tissue fixed with a Cr-mixture. The extraction of KES has been successfullly performed by warming. When the carbol-fuchsin-iodine method (CFJ) is applied to extracted tissue section, the microscopical observation can still reveal a few large drops of violet granules left in the tissue, which are not KES but lipids. Since unsaturatted fatty acids will be well stained by this method, the granules must be differentiated from KES. Pepsin gives no effect on KES; the effect of trypsin cannot be ascertained, because KES has been resolved by a baryta-water mixture employed in its preliminary arrangement. The cow liver nucleotidase digests KES of a raw tissue; but it hardly acts on the tissue KES fixed with a Cr.-mixture. Therefore KES is inactive, if drastic liberation from fat is not carried out previously. With acid phosphatase of human prostate the similar function will be recognized. Ribonuclease cannot serve for the disappearance of KES both from the raw and the fixed tissues. KES does not disappear by the action of crystalline desoxyribonuclease. It is very interest that a remarkable large amount of KES appears as the nucleotides, presumably due to the degradation of DNA in nuclei. Feulgen reaction is negative with KES; while improved Feulgen reaction (Hamazaki) is positive. (That is why KES ought to be extracted when hydrolysis is performed by warm HCl). With tissues fixed by Cr-mixture KES gave a strong ultraviolet absorption at 2600 Å. Since RNA is extracted under this fixation it is not related to absorption. When the CFJ method is imposed upon the same section after the microphotograph, there appear violet granules in accordance with the figure of the absorption. If KES is extracted from the same specimen beforehand by the above mentioned KES-solvents, there takes place no absorption figure. From these results obtained it can be concluded that Hamazaki's ketoenolic substances (KES) is a material chiefly consisting of desoxyribose nucleotides and nucleosides which combine with certain lipids. Though the significance of this substance in living organism is still unknown, it has almost the similar function to folic acid, and, moreover, it can serve as a carrier of molecular oxygen. In case any chronic disturbance of metabolism of this substance should happen, there occurs the formation of wear and tear pigments.

11. An application of reflex microscope to the histochemical investigation of nucleic acid. By KENJI NAKAMURA (*From the Laboratory of Zoology, Faculty of Science, Tokyo University, Tokyo*).

12. Quantitative cytochemistry of nucleic acids. I. Evaluation of "protein effect" in the Feulgen reaction as a source of error in quantitative cytochemical analysis of DNA. By ATUHIRO SIBATANI, MICHIO FUKUDA, HAJIME MATSUDA AND HIROTO NAORA (*From the Institute for Microbial Diseases, Osaka University, Osaka, the Department of Radiology, Osaka University Hospital, Osaka, and the Department Pysics, Faculty of Science, Tokyo University, Tokyo*). The Feulgen reaction *in vitro* developed with dissolved DNA in the presence of an excess Schiff reagent is markedly affected by the co-existence of histone or other proteins dissolved in the reaction mixture. The effect is mediated by the electrostatic combination of histone with DNA, and not by a direct interaction between histone and fuchsin dye. This assumption has been evidenced by the fact that not the concentration of histone, but the ratio of histone which is added to the reaction mixture to DNA, determines the magnitude of the histone effect, which is effaced almost completely by concentrated NaCl. Mean-while, the Feulgenreaction *in vitro* is different from that *in situ*, where DNA, histone and other proteins are in solid state. Prior to he measurement of color intensity with DNA the excess reagent must thouroughly be washed away from the stained material. Consequently, we can not *a priori* expect that the effect of histone and/or other proteins of the cell nucleus upon the histochemical Feulgen reaction is similar to that of proteins in dissolved state upon the Feulgen reaction developed in the test tube. Several attempts have been made to estimate the quantity of fuchsin dye combined with unit quantity of DNA of Feulgen-stained nuclei, macrochemically with the hot perchloric acid extraction. From the data obtained it seems to be unjustified to exclude the possibility that the intensity of the histochemical Feulgen reaction is modified to some extent by variation in type of protein or in the ratio protein to DNA, of in nuclei of different cells; but because of experimental difficulties encountered and not yet overcome, the data are by no means conclusive. With microspectrophotometry of liver cell muclei of a young rat weighting 12.5 g., the extinction coefficient of the Feulgen pigment formed *in situ* can be computed on the basis of DNA concentration in per cent (weight per volume), with an assumption that the absolute DNA content of liver cell nucleus is 6×10^{-12} g. and the loss of DNA by hydrolysis is 10%. The extinction coefficient so obtained is rather close to that of the pigment formed *in vitro* determined by a test-tube experiment conducted simultaneously under comparable conditions. By modifying the composition of color-developing mixture, Feulgen reactions were developed in various intensities, and the extinction coeffificients *in situ* and *in vitro* were calculated in each test. Differences between the respective extinction coefficients of the two series were altogether comparable with those which should occur between the Feulgen reactious *in vitro* with and without histone added, so that it is by no means unlikely that the Feulgen reaction *in situ* is affected by nuclear proteins in essentially the samefashion as is observable with the reaction system *in vitro* in the presence of histone. Therefore, if the ratio of protein to DNA is varied considerably in nuclei of different cell types, the estimation of nuclear DNA content by the Feulgen reaction in conjunction with microspectrophotometry may in volve the " protein error." The presence of such an error may, however, be detected by conducting the histochemical Feulgen reaction with variously modified reaction mixtures which should result in different intensities of the pigment formed. This is inferred from the facts that the effect of histone on the Feulgen reaction *in vitro* consists in the depression or the augmentation of the color intensity according to whether the conditions employed are favorable or unfavorable to the color development with DNA only, and that the magnitude of this effect is enhanced by the increase of histone/DNA ratio. Analysis of this type has indicated that as light difference of r^2E (r: radius of the nucleus in μ, E: extinction at 5461 Å of biconical core of the nucleus) between liver (12.5 g. rat) and adrenal (400 g. rat), as obtained by microspectrophotometry of the Feulgen-stained material, may be due to a true difference of the nuclear DNA con- tent rather than to the " protein error " with nuclei of the identical DNA content.

13. Quantitative cytochemistry of nucleic acids. II. Estimation of nuclear DNA contents of various rat tissues by means of microspectrophotometry. By ATUHIRO SHIBATANI, MICHIO FUKUDA, HAJIME MATSUDA AND HIROTO NAORA. Estimation of the DNA content of various cell nucleiby microspectrophotometry has hitherto been conducted enthusiastically by a number of workers, but the reliability of the results obtained may be questioned, because the techniques employed are subjected to an error of considerable magnitude. With tissues of an adult rat, the values of the relative DNA content of the nucleus expressed in r^2E are as follows: First spermatocyte (leptotene stage) 12.22±0.22; liver cells (main group, preseumably tetraploid) 9.94±0.22; epithelia of convoluted tubules of kidney 5.05±0.18, medulla of adrenal 5.02±0.10; exocrine cells of pancreas (main group, presumably diploid) 4.82±0.19; granular layer of cerebellum 5.16±0.26; and epthelia of esophagus 3.20±0.28; the difference among kidney, adrenal, pancreas and cerebellum being statistically insignificant. Thus, the nuclei of different tissue cells are not necessarily in a strict relationship of geometric progression as regards their DNA contents. Further, the nuclear DNA contents of kidney, adrenal and liver were found to show a systematic alteration during the postnatal growth of the rat. Namely, in kidney and adrenal the DNA content of class I nuclei (presumably diploid) decreases parallelly and markedly with the increase of the body weight from 12.5 up to about 50 g., and beyond this it seems to remain fairly constant. The same holds with liver nuclei. There, the second group of hepatic cell nuclei with higher DNA content (class II, presumably tetraploid), increases in frequency of its appearance during the growth of the animal, and it predominates over the whole nuclear population in rats weighing over 90 g. Now the DNA content of this group increases slightly but steadily with the increase of body weight from 90 up to 400 g. In the livers of rat varying in body weight, the ratio of DNA content of class I nuclei to that of class II nuclei is usually more or less deviated from a strict ratio of 1 : 2. There are hepatic nuclei of still higher DNA contents (class III, probably octaploid), the precise determination of which remains to be performed. Thus, we are now inclined to accept a concept of variability concerning the DNA content of cell nuclei in various rat tissues even within the same ploidy, and to postulate that the variation is, at least in part, systematic during the course of the postnatal growth of the animal.

14. Quantitative cytochemistry of nucleic acids. III. Evolution of nucleic acidt in liver cells of rats during the course of postnatal growth. By ATUHIRO SIBATANI, MICHIO FUKUDA, HAJIME MATSUDA AND HIROTO NAORA. From the foregoing reports there emerges an idea of systematic alteration of nuclear DNA content during the postnatal growth of the rat. However, some question remains about the reliability of the results of microspectrophotometry of Feulgen-stained materials with regard to the possible error due to " protein effect." Therefore, it is desirable to present an additional evidence obtained with an entirely different technique, the gross chemical analysis combined with nuclear count. The mean DNA content of the liver cell nuclei was determined with homogenates prepared by a glass homogenizer essentially in accordance with Cunningham, Friffin and Luck, in order to avoid the error in nuclear count caused by clumping and disruption of the nuclei. The results obtained differed markedly from these author's. The method of Price and Laird, based on the DNA measurement and nuclear count conducted directly with the original homogenate, was proved to be unsatisfactory, because a considerable fraction of nuclei remained in unbroken tissue debris by a milder homogenization, while it is disrupted as a result of a more drastic one, recovery of the nuclear number thus being reduced. Since the mean nuclear DNA centent was expected to vary at different stages of growth it was determined with each liver sample together with total DNA and PAN, and from the figures obtained, total cell number and PNA per cell were calculated. Rats at different growth stages were represented by 5 classes with logarithmic increase of the body weight (12.5, 25, 75, 100 and 100 g.), allowing deviation less than 10%. Three independent determinations were carried out with each of males and females of these groups, every time with a single animal, except for the first two stages, where livers of 5 and 2 individuals of mixed sexes, respectively, were pooled for one determination. In livers of 1.25 g. rats, there was no hematopoietic pictures. Mean nuclear DNA contents (in 10^{-12} g.) of the five stages metnioned were found to be 5.90, 5.09, 9.21, 11.10 and 11.34, respectively; most of the separate determinations at the same growth stage agreed within ±3%. There was no significant difference between ♂ and ♀ at any stage. The above figures are of course average values of lower and higher DNA contents of different hepatic nuclei and of nuclei of Kupffer cells and other cell types of minor abundance. Therefore, a direct comparison of these data with those of the microspectrophotometry is unjustified with different classes of liver parenchyma cells alone. It should be noted, however, that the initial decrease of the nuclear DNA content parallels the picture of the early decrease in nuclear DNA content as revealed by microspectrophotometry. This indicates that the decrease of the nuclear DNA content during the early stages of postnatal growth is highly likely. The data also prove the progressive increase of liver cells in number, mean PNA content of the cell, and mean cell mass throughout the whole period of the growth examined.

15. A review on the chemistry of nucleic acid. BY KATASHI MAKINO (*From the Department of Biochemistry Kumamoto University School of Medicine, Kumamoto*).

16. Studies on nucleic acid metabolism in kidney tuberculosis. I. BY SHUN SHIGEMATSU AND HIROSHI TAKAHASHI (*From the Clinic of Dermato-urology, Kurume Medical College, Kurume*).

17. Ribonucleic acid and protein synthesis in the growing oocyte of *Triturus pyrrhogaster*. BY SYOZO OSAWA AND YUJIRO HAYASHI (*From the Biological Institute, Faculty of Science, Nagoya University, Nagoya*). The intracellular metabolism of RNA in growing cells is said to correlate with the intracellular synthesis of proteins. As the first step to clarify this relationship, cytochemical detection as well as quantitative estimation of RNA and protein during oogenesis were performed. *Histochemical Detection*: In the young oocyte cytoplasmic the staining with basic dyes (RNA) was very strong. Nucleoli showing intensive basophilia were attached to the inner wall of the nuclear membrane. With the growth of oocyte, the stainability decreased gradually, and nucleoli were more and more gathered in the centre of nucleus. As Brachet repeatedly stressed, these results impressed us as if the cytoplasmic RNA of the growing oocyte were rapidly decreasing during oogenesis. But the volume of the grown oocyte was over 100 times as great as that of the young one. The decrease in concentration judged by the lowered stainability will not necessarily mean the absolute decrease in the amount of RNA of the growing oocyte. *Quantitative Estimations*: The amount of RNA per oocyte increased progressively with its growth, but from certain stage afterwards, the value remained constant in spite of the increase in volume. RNA concentration was shown to fall rapidly and then decline slowly. From the quantitative results it can be assumed that the decreasing RNA content in the growing oocyte observed by the histochemical study may be nothing but a representation of "decreasing of concentration," *i.e.*, the diluting effect of other increasing components but not a decrease in the absolute amount of RNA per cell. In oocyte of the dessending phase in its absolute amount of RNA, many nucleoli were attatched to the inner wall of the nuclear membrane, intensely stainable with basic dyes and appeared full of activity. Larger sized oocytes in which RNA per cell no more increased in spite of further growth, contained many degenerating nucleoli only in the central portion of the germinal vesicle; they were never so basophilic and no more attatched to the nuclear membrane. Thus, these facts suggest a possibility that the nucleoli may play a rôle in the synthesis of RNA of cytoplasm. Quantitative measurement of total protein-N by using Schneider residue linearly with the increase of cell volume. If the appearence of the germinal vesicle in larger sized oocytes is in fact a representation of the inactivated state of nucleus, a considerable amount of protein would be synthesized with no bearing on the nuclear activity.

18. Study on the metabolism of nucleic acids in rat liver using radioactive phosphorus. By TAIJI SHIMIZU (*From Department of Biochemistry, Niigata University School of Medicine, Niigata*). Nucleic acids were extracted from the liver of the rats injected with P^{32} in the form of Na_2HPO_4, and the turnover rates of phosphorus of nucleic acids were measured one hour after the intection. RNA and DNA were then separated by the slight modification of both Schmidt-Thann-hauser's and Schneider's methods. The incorporation of P^{32} into RNA and DNA was observed for a short time (one hour) after the injection, and the turnover rates of RNA and DNA P (Sacks' relative specific activity) were observed to be depressed in thiaminedeficient rats.

19. Effect of x-ray on the nucleic acid contents in the cells. By KISHI MIURA, HAJIME MATSUDA, KIYOHARU AKITA and YONEMASA KASUMI (*From the Clinic of Radiology, Okayama University, Okayama*).

20. Nucleic acid metabolism in patients with uterus cancer. By TSUNEO HANAOKA (*From the Department of Biological Chemistry, Okayama University Medical School, Okayama*). The uracil contents in urine of healthy women ranged from 5 to 80 γ%. (average 30 γ%), whereas that of the individual suffering from uterus cancer was 25 to 214 γ% (average 102 γ%) before treatment, but after extirpation of the cancerous tissue, the value was lowered to 2 to 13 γ% (average 7 γ%). Patients receiving radiological treatment also displayed a low urinary uracil value ranging from 2 to 13 γ% (average 5.7 γ%). Therefore, it can be concluded that, as compared with healthy Aomen, cancer patients excrete much more uracil as a result of abnormal nucleic acid metabolism in the cancerous tissue. On the other hand, paper chromatographical analyses were performed with each 5 ml. of uracil and thymin fractions from 5 liters of urine collected from healthy, pretreated uterus cancer patients and uterus cancer patients treated radilogically. In cancerous patients preceding treatment, both uracil and thymin were demonstrated remarkably, while in patients under radiological treatment, both compounds may be detected although in small amounts. However, in healthy women uracil was rarely detectable, and thymin was quite absent. This fact substantiates the theory that DNA metabolism in cancerous tissue is accentuated.

21. **A further evidence that lymphocyte may serve as the origin of desoxyribonucleic acid.** By KEN OONO and SETSUKO KOBAYASHI (*From Tokyo Agricultural and Technological College, Tokyo*).

22. **Purification and some chemical properties of toxohormone produced by cancer.** By SABURO UGAMI, KAZUO KURETANI and KAZUMI IGARASHI (*From the Scientific Research Institute, Tokyo*). For the purification of toxohormone, a characteristic toxin produced by cancer, having the action of reducing the liver catalase activity, the isoelectric precipitation method was applied to non heat-coagulable fractions of rat Yoshida sarcoma and human reticulosarcoma. Fractions obtained by once precipitation at around pH 2.8 had approx. the same potency as those prepared by cupric precipitation method of Nakahara and Fukuoka. By repeating the isoelectric precipitations the potency is likely to rise. The preparations by cupric and isoelectric precipitation methods contained appreciable amount of RNA, which was further fractionated into RNA and non-PNA fractions by boiling with 5% CCl_3COOH, both of which had, however, no activity to reduce liver catalase. Thus it is assumed that toxohormone may be destroyed by this fractionation. By $CHCl_3$ gel method, however, the PNA fraction was inactive but the non-PNA active. Thus it seems unlikely that the principal part of toxohormone is PNA.

23. **The in vitro production of catalase by rat liver slices and hemogenates.** By SABURO UGAMI, KAZUO KURETANI and KAZUI IGARASHI (*From the Scientific Research Institute, Tokyo*). The rat liver slice was well rinsed in normal saline to remove the contaminants as completely as possible, and kept in various media at 37°; and the catalase activities in the medium and tissue were measured 0, 1.5 and 3.0 hours after the incubation. Under appropriate conditions, *i.e.*, aerobically in Krebs-Henseleit bicarbonate solution supplemented with ATP, the contents of amino acids and glucose and the total catalase activity (medium+tissue) increased progressively 3～4 folds in 3 hours' incubation. The necessities of ATP and oxygen may suggest that the increase in total catalase content represents a real synthesis of catalase. Both slice and homogenate were proved to be capable of producing catalase in the presence of ATP. Acetone-dried powder of the slices, however, had no catalase producing action. In comparison of the result of the sedimental fraction with that of non-sedimental fractions of the homogenate, it is suggested that the catalase producing action in question may be located on the macroparticles of liver cells.

24. **The effect of chemicals of the digitalis group on the nucleic acid of *several organs*.** By MASAYUKI NAKASTUKA and HARUE ARATANI (*From

the Department of Pharmacology, *Hiroshima Medical College*, *Kure*). In order to clarify the mechanism of action of the chemicals belonging to digitalis group, the tissue culture of the cardiac muscle of a chick embryo was continuously administered with these chemicals. With the cardiac muscles of frog and cat, the action of the chemicals was also examined histologically. During this process, the inclination of DNA-increase in the cardiac cells of heart atria was confirmed. As chemicals, digitoxin, g-strophanthin, helleborein, and digicorin were administered continuously for 10 days (initial trial—lethal does 50; final stage—lethal does) to a frog, and the nucleic acid contents in heart, liver and kidney were determined. (1) DNA: In heart, there was a very slight, but less significant increase in DNA, but in kidney g-strophanthin and helleborein increased DNA more effectively than digitoxin and digicorin; in liver, no marked change in DNA was observed, but digitoxin and digicorin rather decreased DNA to a certain extent, while g-strophanthin slightly increased it. (2) RNA: In heart, RNA increasd slightly; in kidney, RNA scarcely changed, but it rather decreased, and helleborein and g-strophanthin caused a greater decrease; in liver, there was generally no change or a tentative decrease in RNA, except for a slight increase by helleborein. The above change in RNA contents were a little more distinct than that of DNA. Among the chemicals, helleborein resulted in the greatest change, with digitoxin and g-strophanthin following, while digicorin showed the least change. (3) The ratio, RNA/DNA: In heart the ratio rarely changed; in kidney there occurred no change in the ratio by digitoxin and digcorin, but rather a decrease by g-strophanthin and helleborein; in liver, digot oxin or digicorin nardly changed the ratio, g-strophanthin increased it but helleborein rather decreased it. In summarizing these results, the changes in nucleic acids in frog organs were greater in the order of kidney, liver, heart.; and the grade of action of the chemicals were stronger by the order of helleborein, g-strophanthin, digitoxin, digicorin. It can be surmised that the degree of toxicity and secondary reaction may be connected with the degree of change in the necleic acids of the kidney and liver. In case of heart, in which the change in nucleic acids is smaller than the other organs, and nucleic acid contents are increased by all chemicals, it may be assumed that the increase in nucleic acids is an important factor in regards to the true nature of action of these chemicals.

25. Studies on nucleic acid metabolism of fowl erythrocyte nuclei. By SUSUMU WATANABE and TETSUO MONZEN (*From the Department of Pathology*, *Hiroshima Medical College*, *Kure*). The destaining of erythrocytes has been known to be caused by the application of 0.1—0.5% sodium cholate to the methanol-fixed blood smear of the fowl, where the stainability of erythrocyte nuclei gradually diminishes until the nuclei become almost completely unstained. The histochemical examinations by the ultraviolet microscopic photograph, Feulgen reaction and methyl-green-pyronine staining will prove that such a destaining process may be induced by the removal of DNA within the cuclei. While investigating a specimen form the lymph node treated with the similar procedures, the authors have happened to notice that, in the hemorrhagic field, the destaining process of lymphocyte neclei appears to be accentuated to a considerable degree; on the other hand, the blood serum shows more effective destaining action than cholate solution. In conclusion, it has been confirmed that the sera of the various vertebrata and nonvertebrata contain the active substance, having the ability to remove the stainable substances from the fowl erythrocyte nuclei after the methanol-fixation of blood. This active substance will probably be identical with dexoxynucleodepolymerase of Greenstein. Furthermore, this substance is shown to have the similar activity to the pusified crystalline desoxy ribonuclease. The enucleation process of the fowl erythrocyte nuclei after the fixation is considered to consit in removing DNA in early stages and later disposing of the stroma.

26. Phosphorus metaborism in *Staphylococcus*. II. Inhibition of nucleic acid metabolism by nureomycin and terramycin. By YOSHIAKI MIURA, YASUKICHI NAKAMURA, YASUO YOSHIZAWA AND TAKAE KOMEJI (*From the Department of Biochemistry, Faculty of Medicine, Tokyo University, Tokyo*).

27. Studies on the reproductiou of bacterial virus. On the nucleic acid synthesis in ultraviolet ray-injured *Escherichia coli*. By ITARU WATANABE, KENSHI SUZUKI AND MASAYOSHI NAKAMURA (*From the Institute of Science and Technology, University of Tokyo, Tokyo*). It is generally believed that viruses require living host cells for their growth. Recently it has been shown that bacterial viruses can multiply in the presence of *Escherichia coli* in advance rendered incapable of forming visible clolonies with the irradiation of ultraviolet ray. In order to clarify the metabolic activities of these ultraviolet ray-injured cells, the nucleic acid-synthesizing power of the irradiated cells was compared with that of the untreated cells. Lag phase cells of *Escherichia coli* (strain B) suspended in physiological saline solution were irradiated with ultraviolet ray for an appropriate timd and resuspended in fresh nutrientbroth at 37°. Half of the suspension was immediately inoculated with T_2 virus (4 to 8 virus particles per cell.) After 80—90 mimutes the increments in DNA and PNA contents of four cultures—namely, thh untreated *coli*, the untreated *ooli*+T_2 virus, UV (ultraviolet ray treated)-*coli*, UV-*coli*+T_2 virus—were determined. (a) The treated cells of *E. coli* gradually lost their DNA-synthesixing power at first accompanying no alteration of their capacity of PNA-synthesis, when they were irradiated for relatively short time. When irradiated for a long time, the DNA formation was almost completely blocked and the PNA-synthesis was also reduced to 10—20% of the control. (b) When infected with T_2 virus, the UV-*coli*, having neither DNA-nor PNA-synthesizing capacity for their own nucleic acids, were still able to produce virus DNA to the same extent as the untreated *coli*. (c) The virus DNA-synthesizing power of *E. coli* was lowered only after strong irradiation; therefore, their PNA-forming capacity was also completely blocked. From these results obtained, it is suggested that the capacities to synthesize bacterial DNA, bacterial PNA and virus DNA may be considerably independent of each other in the cell of *F. coli*.

28, P^{32}-labeled endotoxin of *Pseudomonas aeruginosa*. By J. YUZU-

RU HOMMA, TADAO KATSURA, SEIGO HOSOYA, YOSHIO MIYAZAKI, KENJIRO KIMURA AND NOBUFUSA SAITO (*From the Institute for Infections Diseases, and the Department of Chemistry, Tokyo University, Tokyo*). The purified endotoxin of *Ps. aeruginosa* isolated from the culture filtrate has been proved to be mainly composed of nucleprotein. For isotopical investigation of the endotoxin, the cells of *Ps. aeruginosa* were cultivated at 37° for 30 hours by shaking method in the synthetic medium consisting of 2% Na glutamate, 0.5% glucose, 0.01% glucose, 0.01% $MgSO_4$, 0.005% phosphate containing 16—400 μ Curie P^{32}. From the culture filtrate the radioactive endotoxin was isolated and it was shown to be quite homogeneous eletrophoretically. Chemical analyses gave 9.5%, N, 2.9% organic P, but 0% inorganic P, which indicated the existence of P^{32} as molecular compnents in the endotoxin. The P^{32}-labeled endotoxin was foung to be identical with the non-labeled endotoxin in respects to their properties, *i.e.*, the precipitin reaction, Schwartzmann phenomenon and toxicity. The distribution of P^{32} in the mouse body in 1—5 hours after the intravenous administration of the P^{32}-labeled endotoxin was the strongest in liver, decreasing by the order of intestinall wall, lung, bones. The values of radioactivity were variable with the time after the injection. The liver homogenate of the injected mouse was fractionated by Schneider's method, and the radioactivity of P^{32} was determined in the nucleic acid and phosphoprotein fractions. The endotoxin was also detected by preciptin reaction in the liver extract at least till 5 hours after the injection; the radioactivity in liver is considered to be mainly due to the P^{32}-labeled endotoxin itself. The specific pathological changes were most remarkably observed in liver (cloudy swelling of cells, hydropically swollen nuclei, pyknosis and karyolysis in some nuclei, the increase in binuclear cells, sometimes miliary-sized nectroic foci, swollen and increased stellate cells, and mild and diffuse leucocytic infiltrations). The weak changes were also found in lymph nodes (enlarged follicles), and spleen (increases in plasma and reticular cells), frequently in myocardium (granular degeneration); and in lungs (a slight degree of interalveolar pneumonia); but no special change was observed in adrenals. In conclusion, thhse results of isotopical and chemical analyses as well as of pathological examinations will indicate that the injected endotoxin to mice may be mainly fixed in liver.

29. Studies on desoxyribose. I. By KIYOHIKO OHTA (*From the Department of Biochemistry, Kumamoto University School of Medicine, Kumamoto*).

30. Molar ratio of purines and pyrimidines in yeast nucleic acid. By MASAO UCHIDA (*From the Department of Biochemistry, Kumamoto University School of Medicine, Kumamoto*). For the determination of guanine, the author has proposed a method, consisting of the precipitation of purine by silver method and by the hypoiodite method of Willstatter and Schudel. From the analytical values of N, P, amino-N (van Slyke), purine-N (copper bisulfite method), and guanine, it is suggested that the molar ratio of adenine, guanine, cytosine and uracil in yeast nucleic acid (Marck) is equimolecular.

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